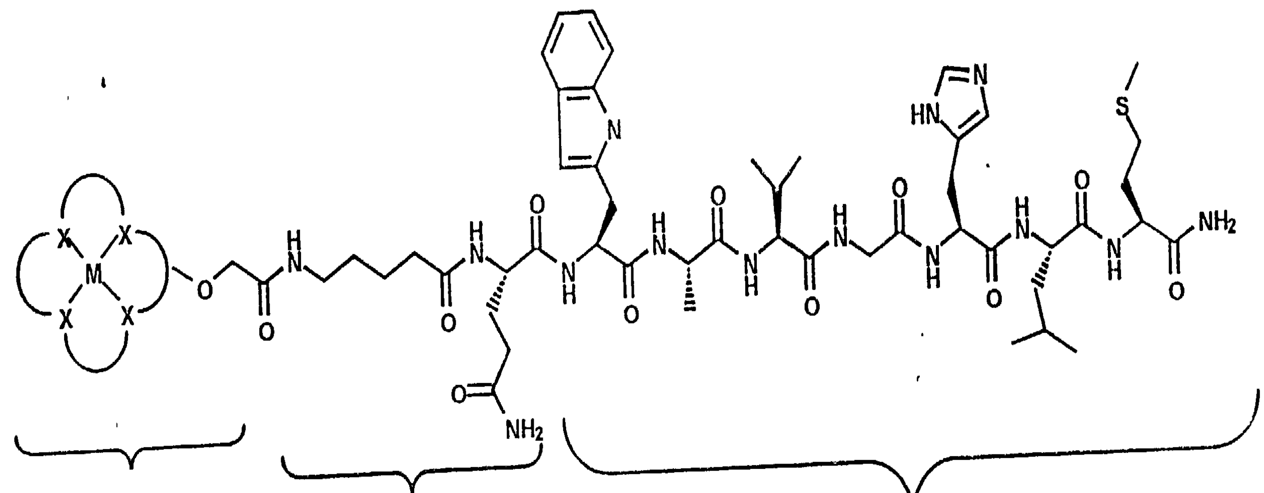
SCANNED,# 14

Radiometal Conjugate



RMC
Radiometal Chelate

SG
(Spacer Group)

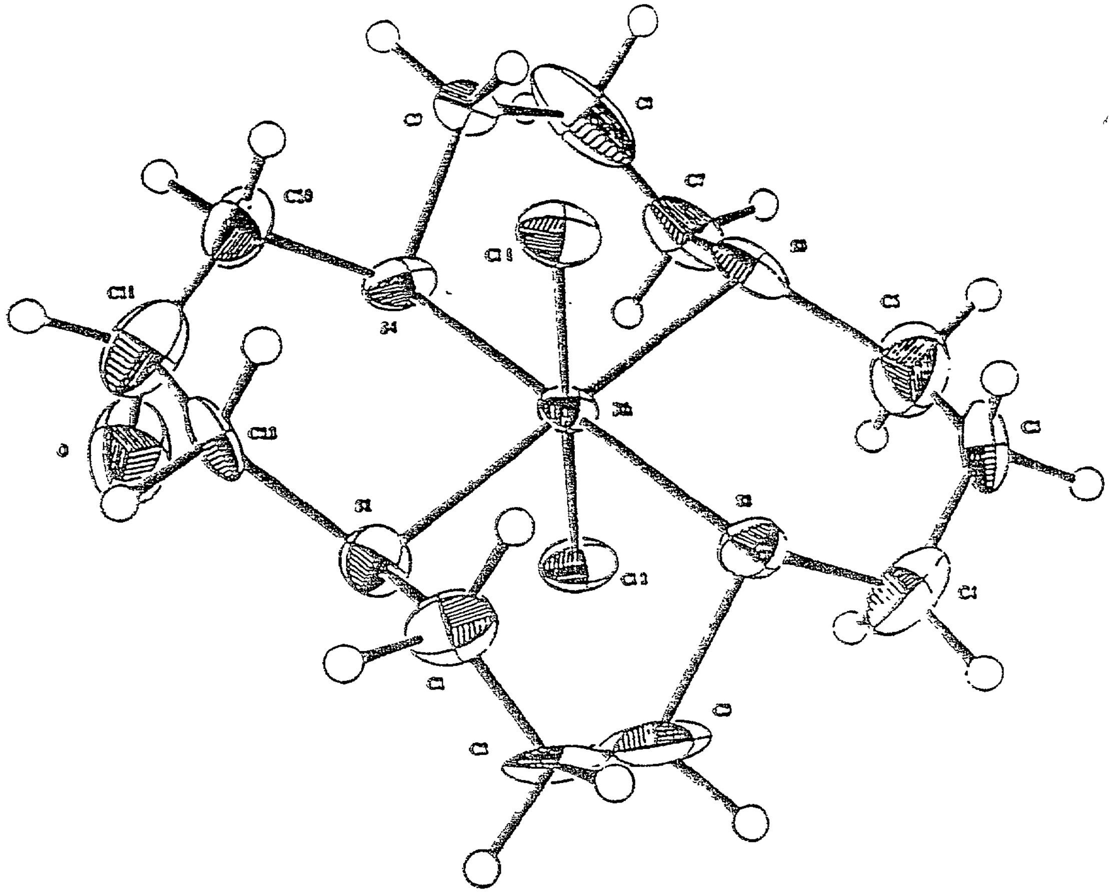
BBN(8-14)
Bombesin Binding Region

Radiometal conjugate of a BBN analogue that hashigh affinity for GRP receptors.

RMC=Radiometal chelate, where M=^{99m}Tc, $^{186/188}Re$, ^{105}Rh and X=chelating atoms.

SG=Spacer group or linker that covalently attaches the chelate to the N-terminal end of the BBN binding region (BBN_{BR})

Figure 1



ORTEP Drawing of $\{Rh[16]aneS_4\text{-}olCl_2\}^+$

Figure 2

10205049.102460

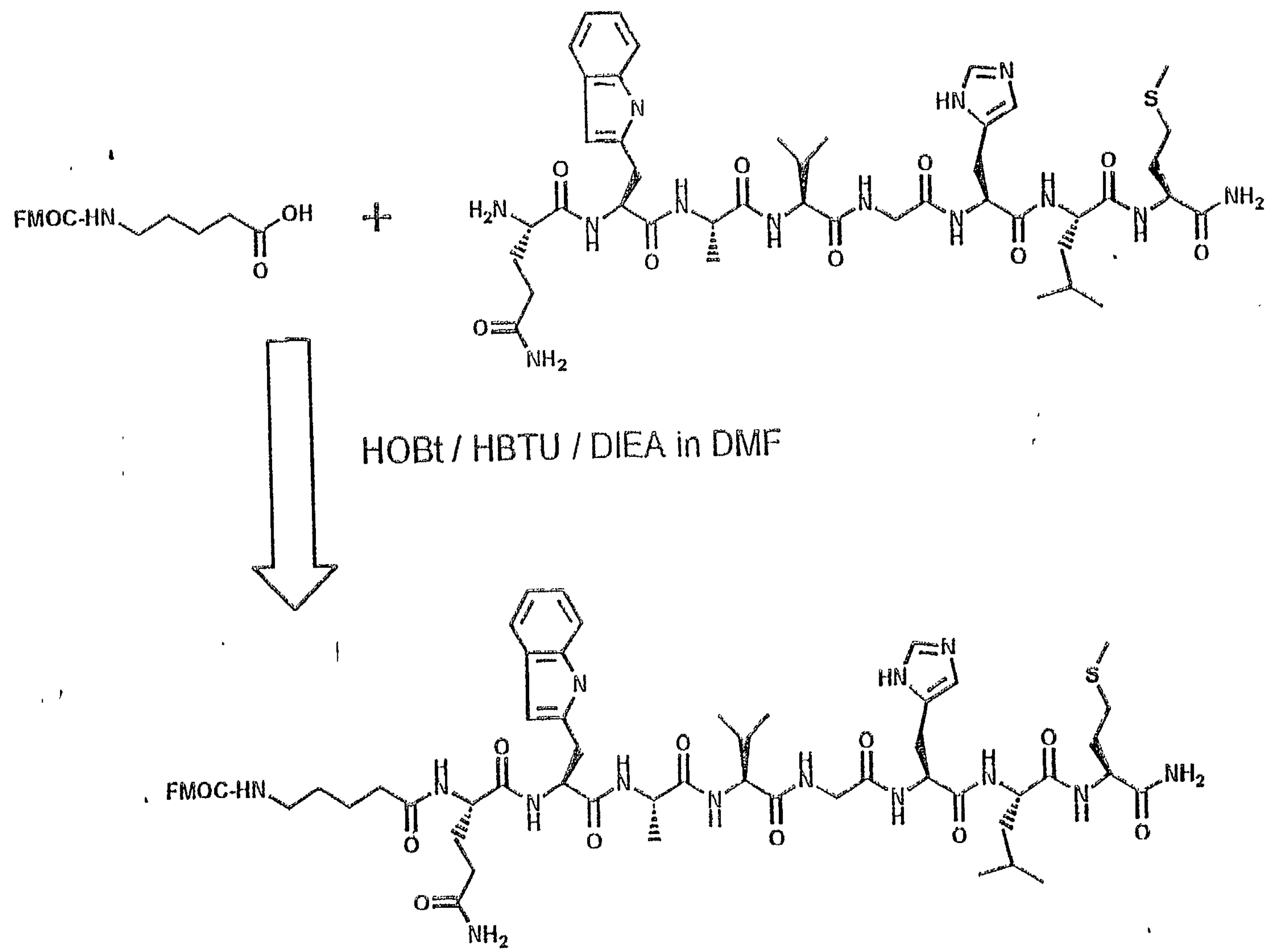


Figure 3

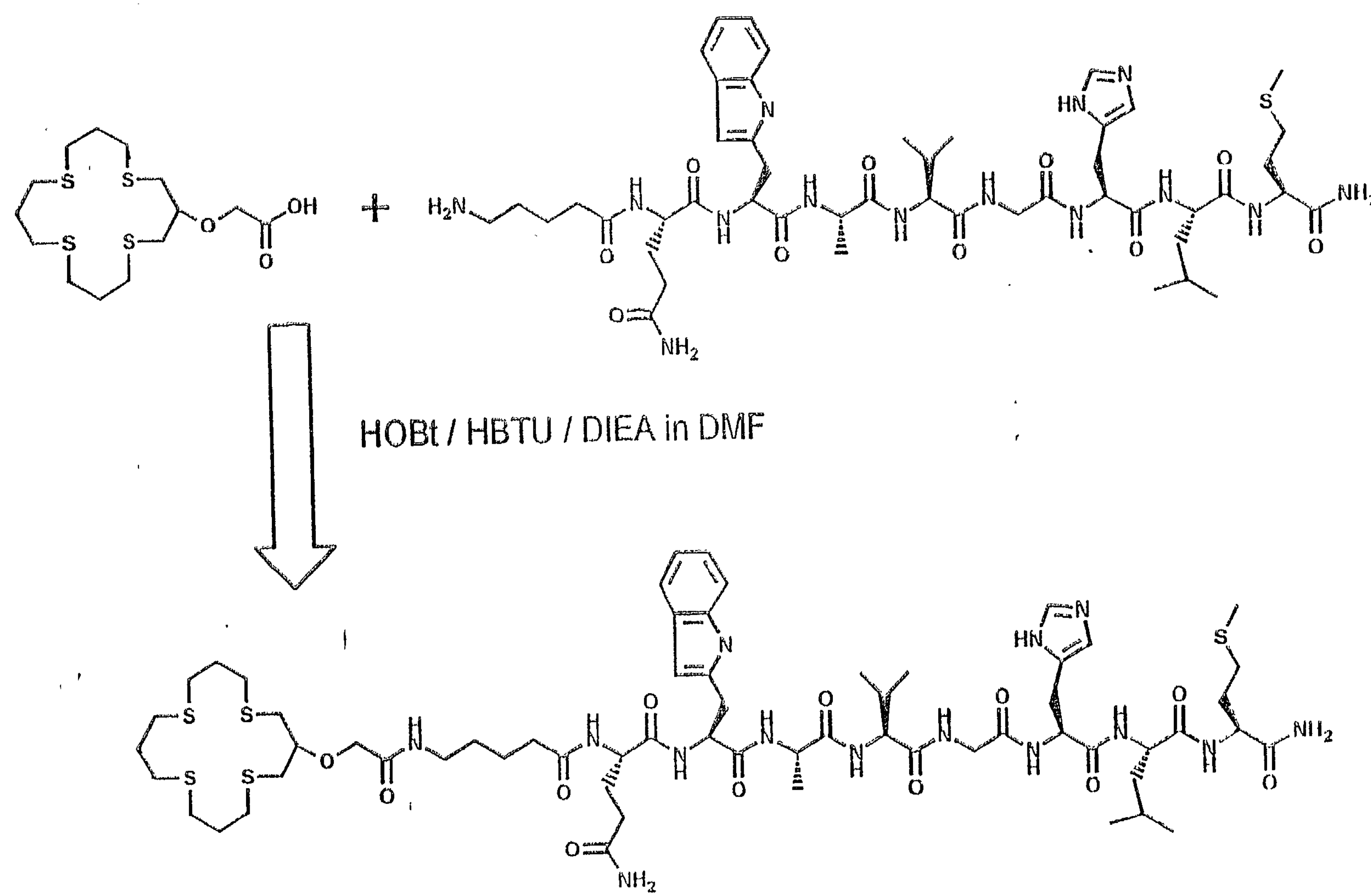
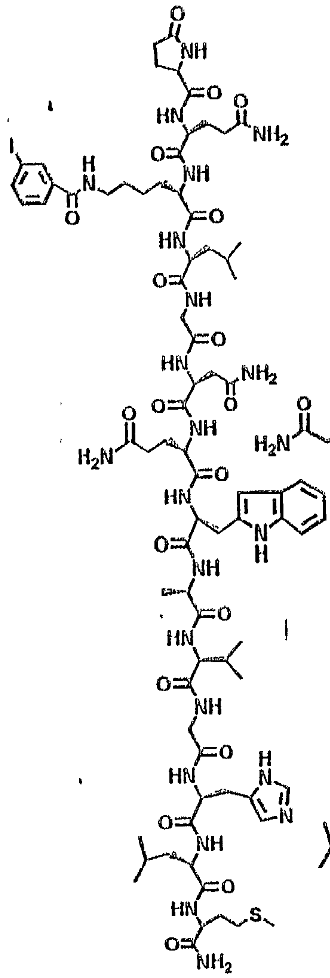


Figure 4

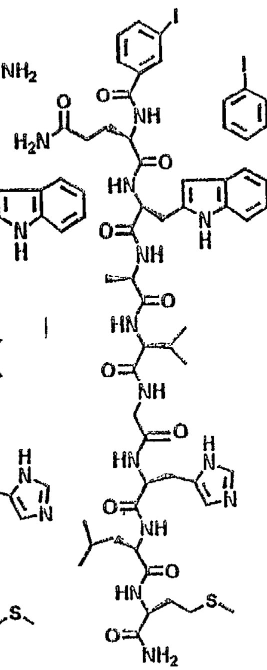
Iodinated Bombesin Analogues

mIP-Lys^3-BOMBESIN



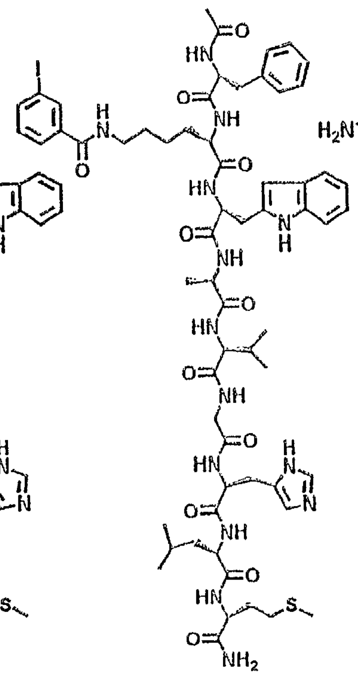
IC_{50}=2.13

BBN-18



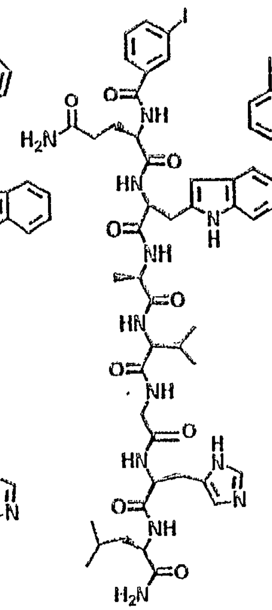
IC_{50}=5.80

BBN-16i



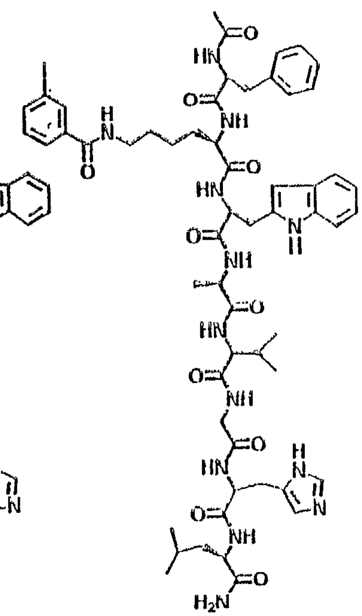
IC_{50}=18826

BBN-9



IC_{50}=0.14

BBN-15i



IC_{50}=>40,000

Figure 5

Tethered Bombesin Analogues

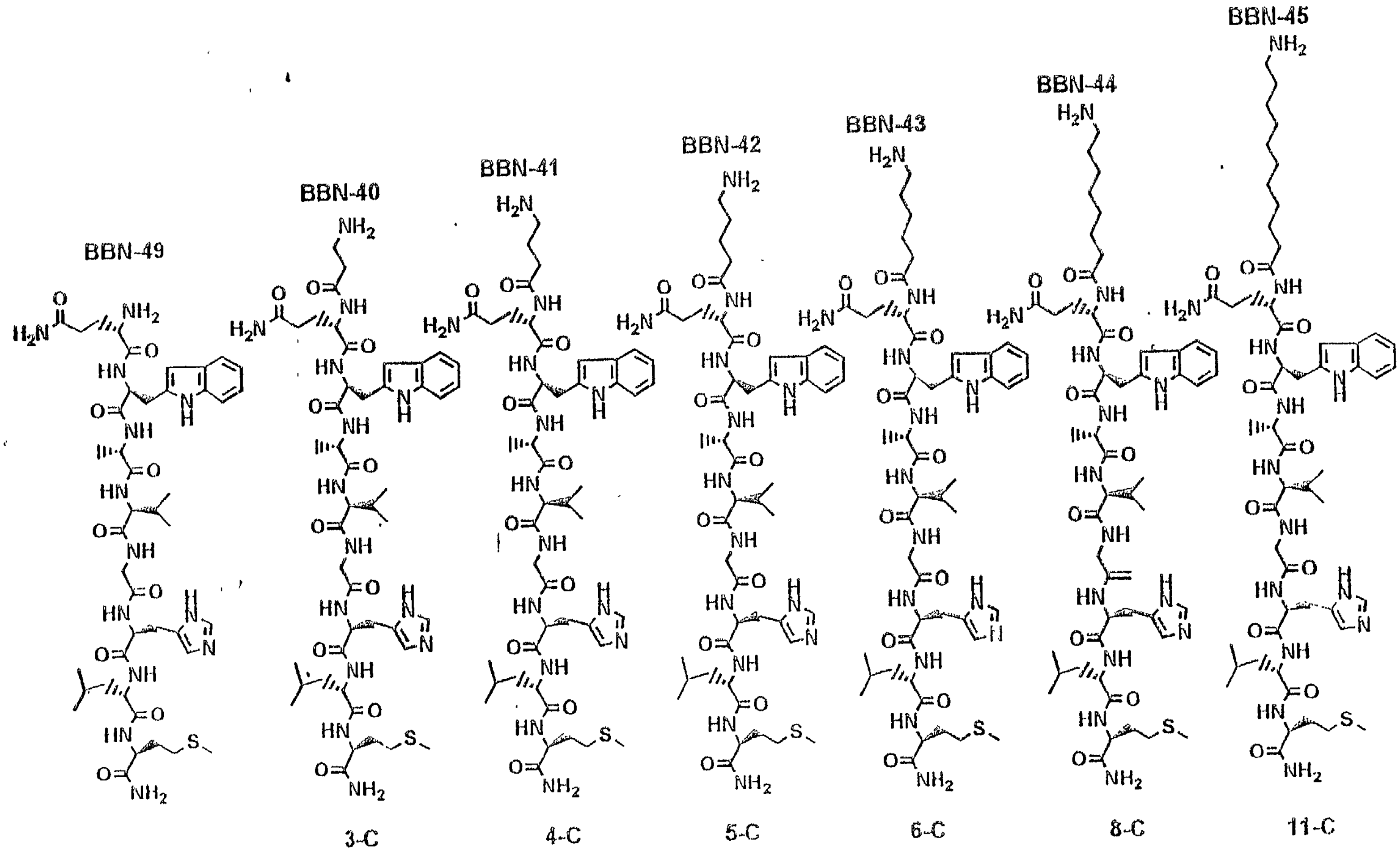
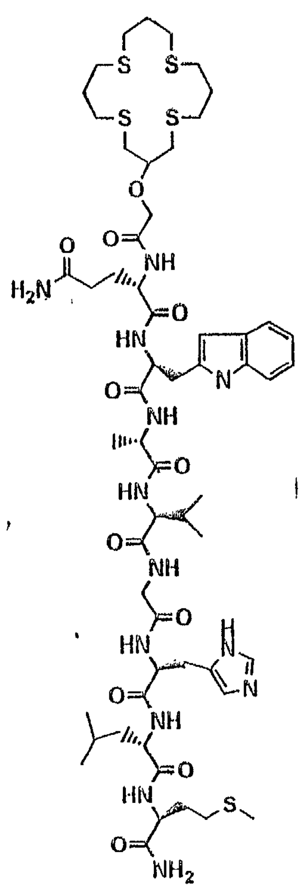


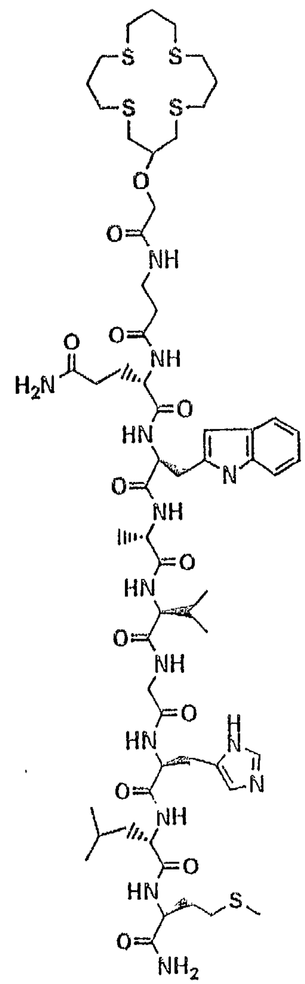
Figure 6

[16]aneS$_4$ Bombesin Analogues

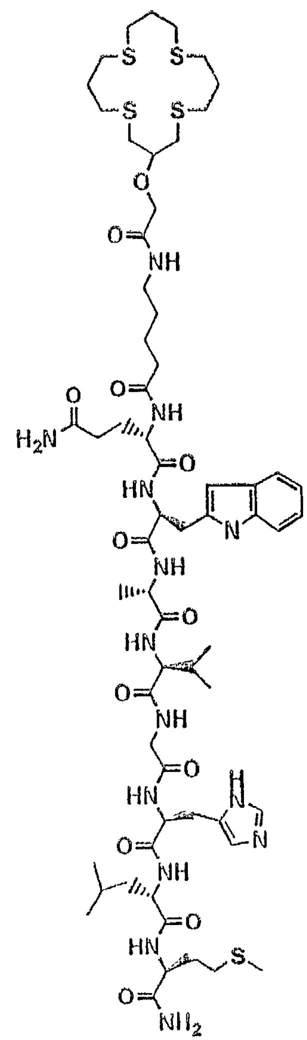
BBN-22



BBN-46



BBN-37



BBN-47

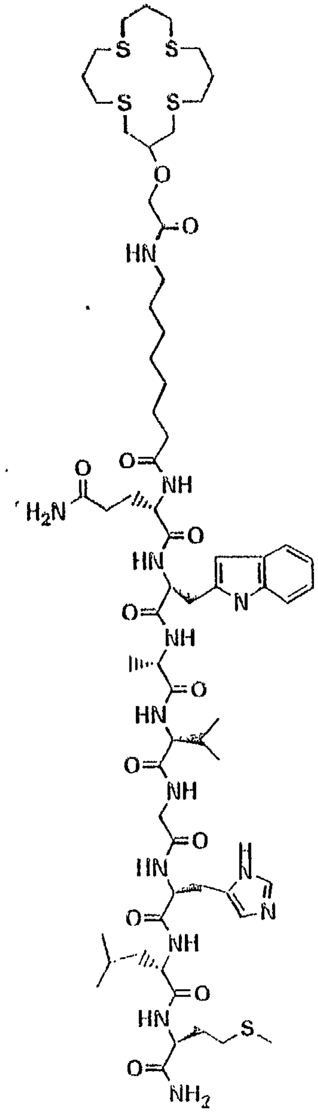
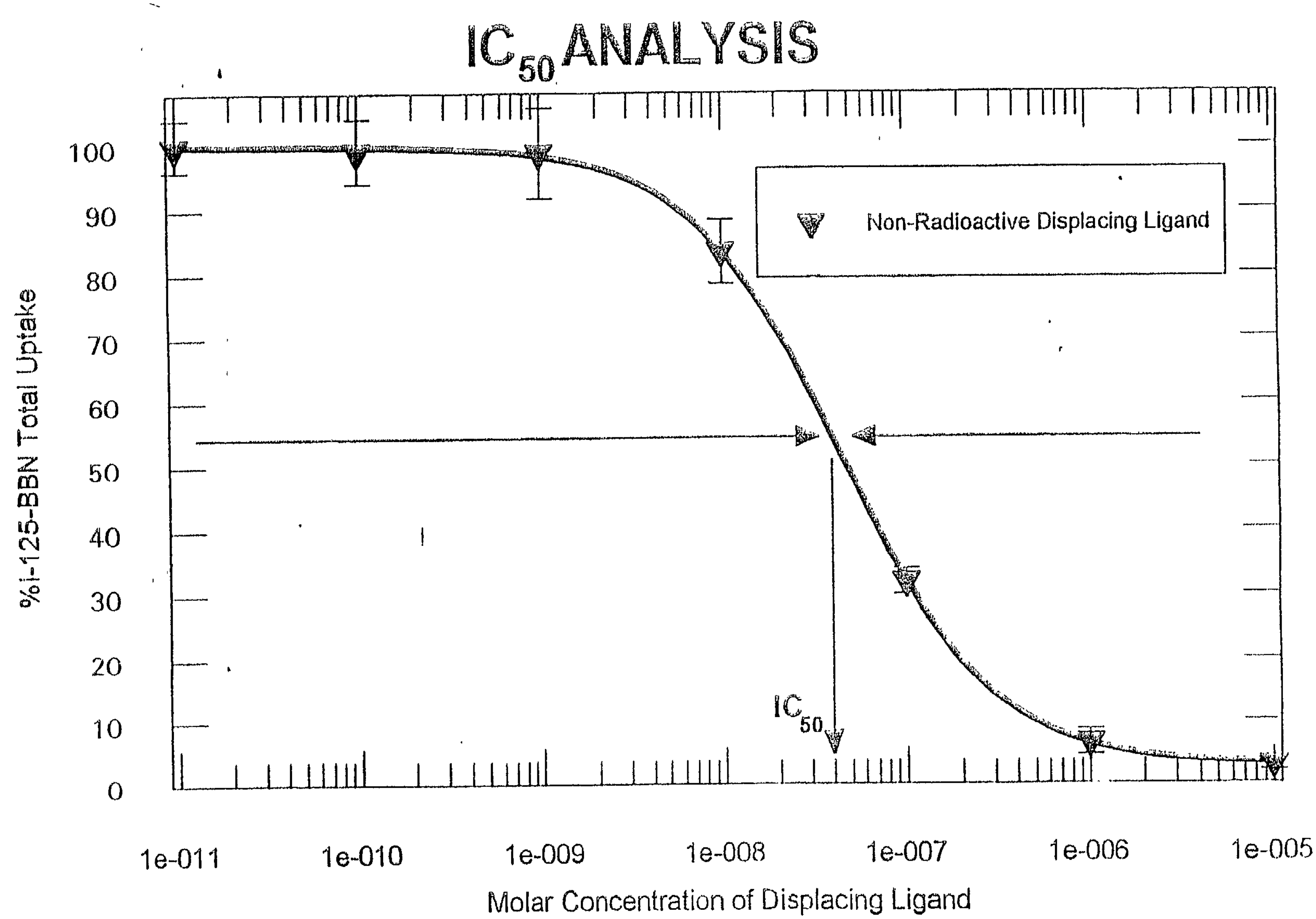


Figure 7

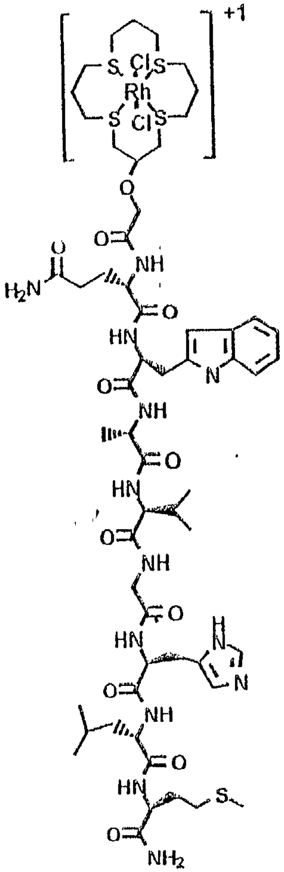


IC50 ANALYSIS
100
90
80
70
60
50
40
30
20
10
0
%I-125-BBN Total Uptake
Non-Radioactive Displacing Ligand
IC50
1e-011
1e-010
1e-009
1e-008
1e-007
1e-006
1e-005
Molar Concentration of Displacing Ligand

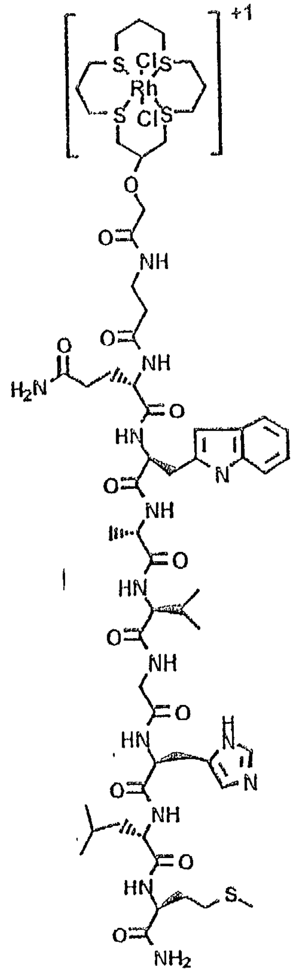
Figure 8

Rhodium-[16]aneS_4 Bombesin Analogues

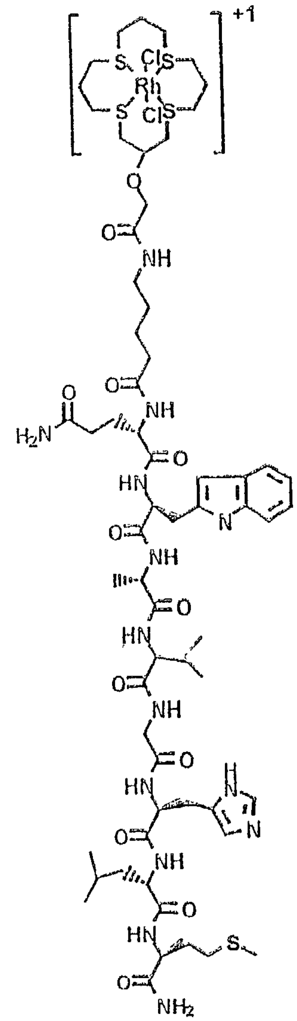
BBN-22



BBN-46



BBN-37



BBN-47

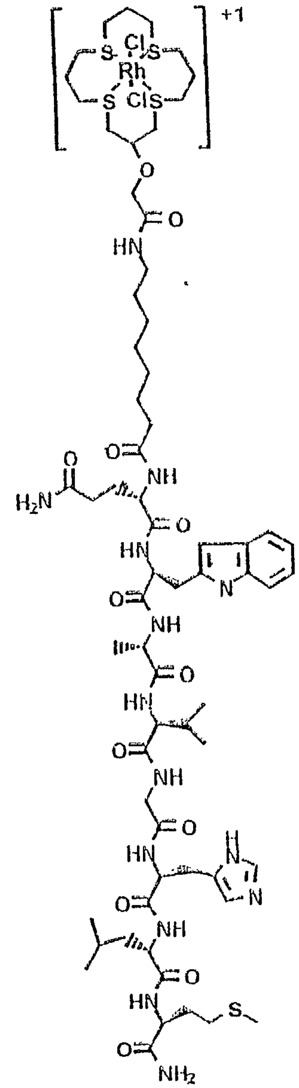
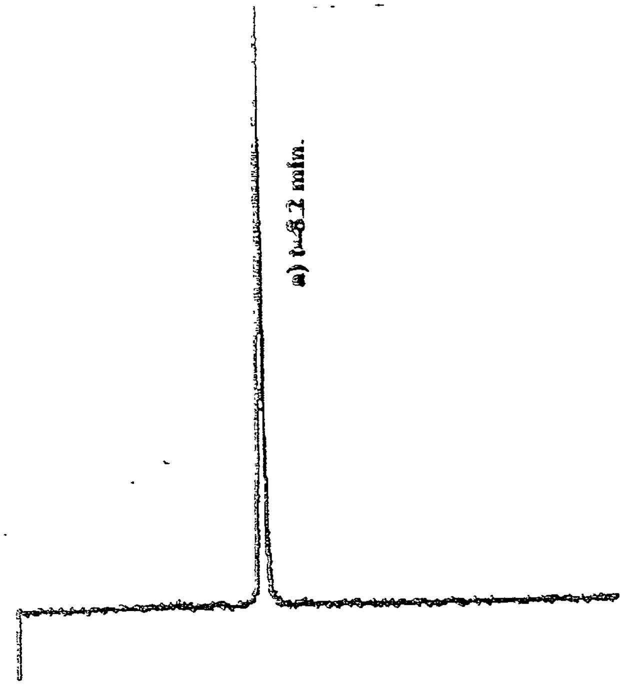
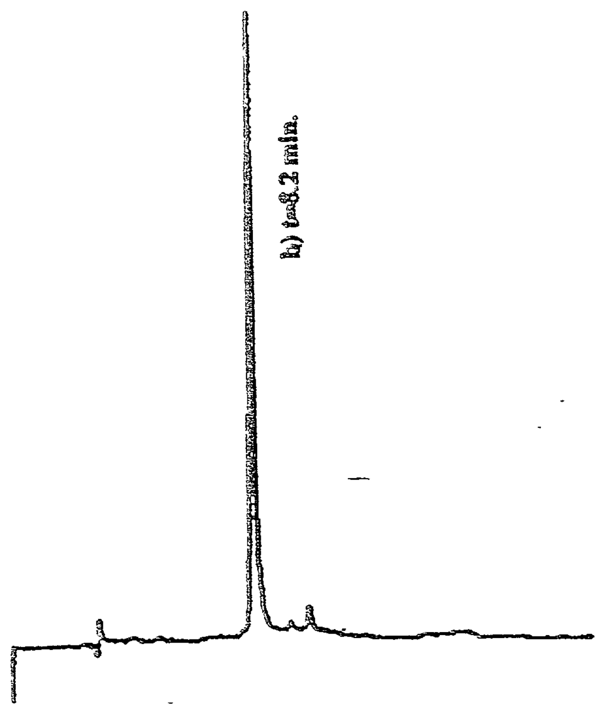


Figure 9

A.

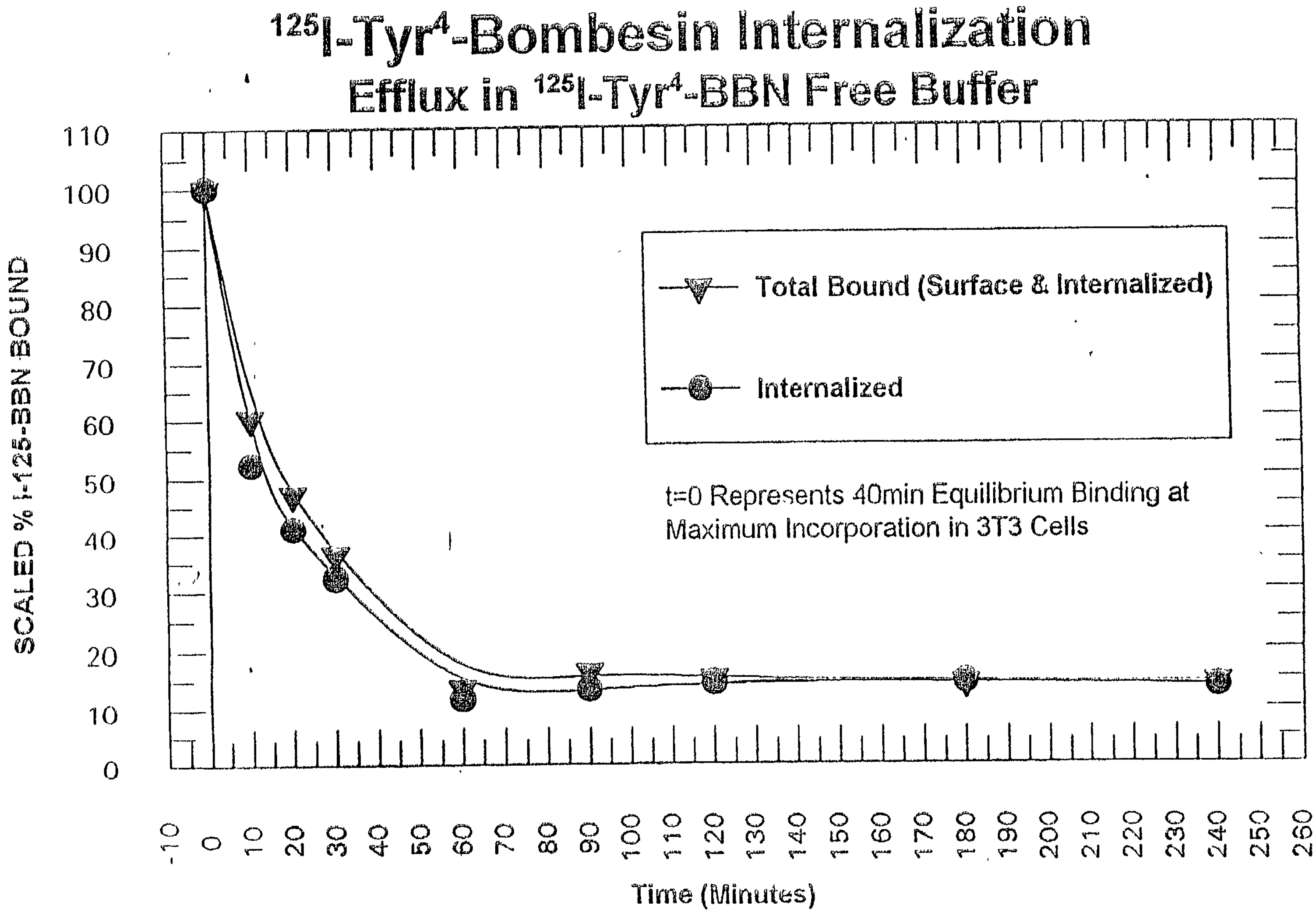


B.



HPLC Chromatogram of Rhodium-BBN-37
Top: $^{105}RhCl_2$-BBN-37
Bottom: $RhCl_2$-BBN-37

Figure 10



125I-Tyr4-Bombesin Internalization
Efflux in 125I-Tyr4-BBN Free Buffer
SCALED % I-125-BBN BOUND
110
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
Time (Minutes)
Total Bound (Surface & Internalized)
Internalized
t=0 Represents 40min Equilibrium Binding at
Maximum Incorporation in 3T3 Cells

Figure 11

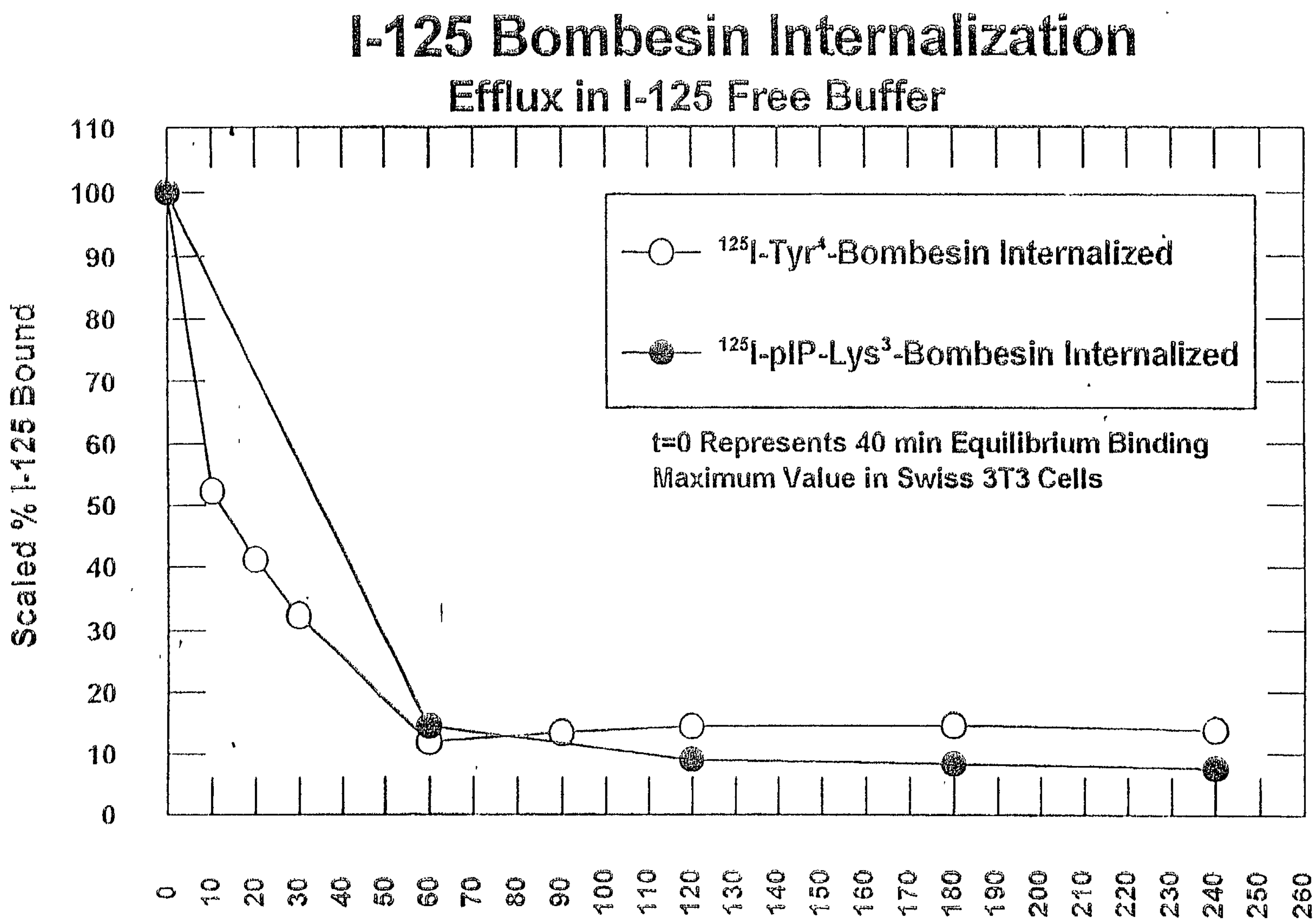

I-125 Bombesin Internalization
Efflux in I-125 Free Buffer
125I-Tyr4-Bombesin Internalized
125I-pIP-Lys3-Bombesin Internalized
t=0 Represents 40 min Equilibrium Binding
Maximum Value in Swiss 3T3 Cells
Scaled % I-125 Bound
110
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
Time (Minutes)

Figure 12

Efflux of ^{105}Rh-BBN-37 in Swiss 3T3 Cells

(Normalized Data)

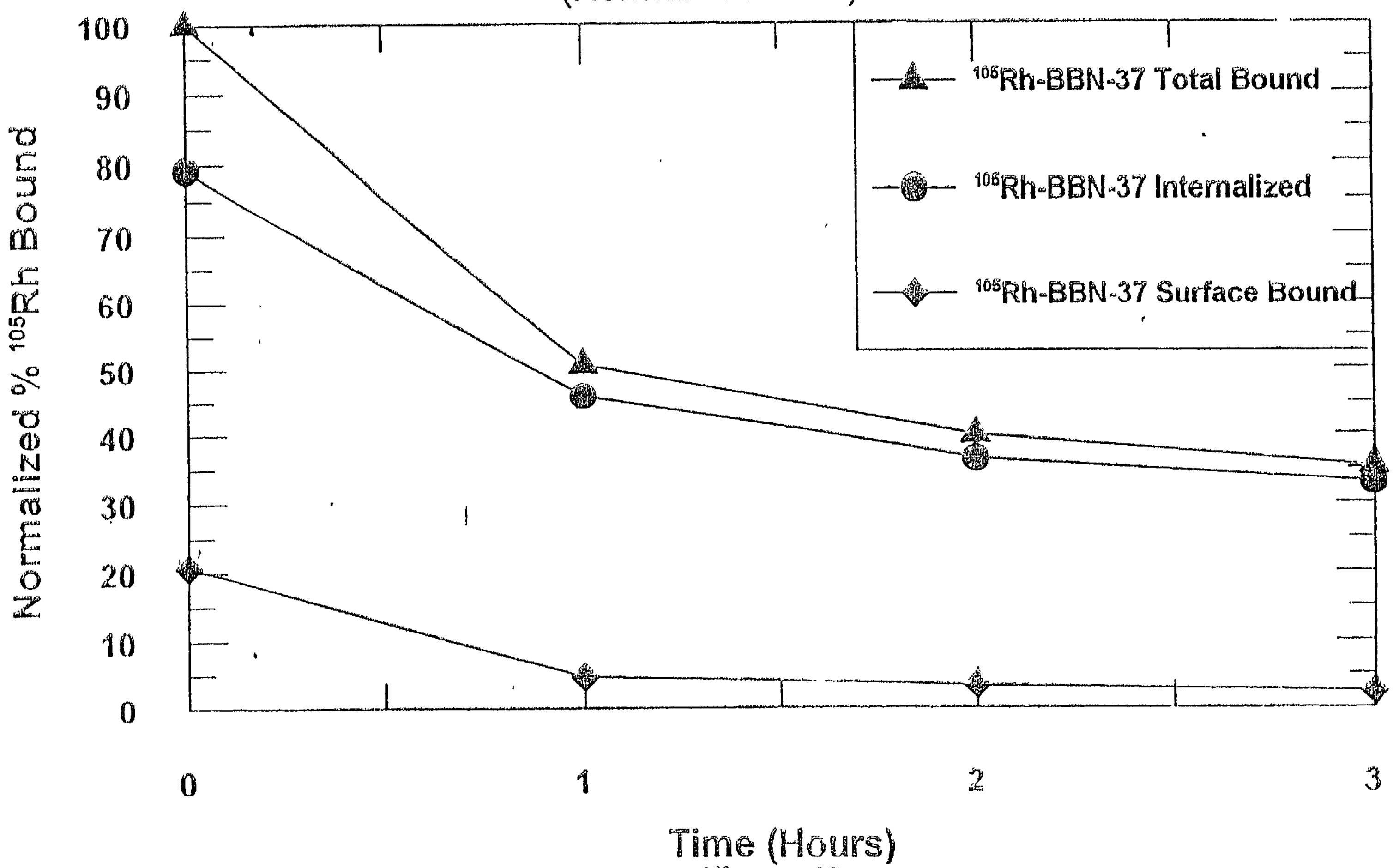
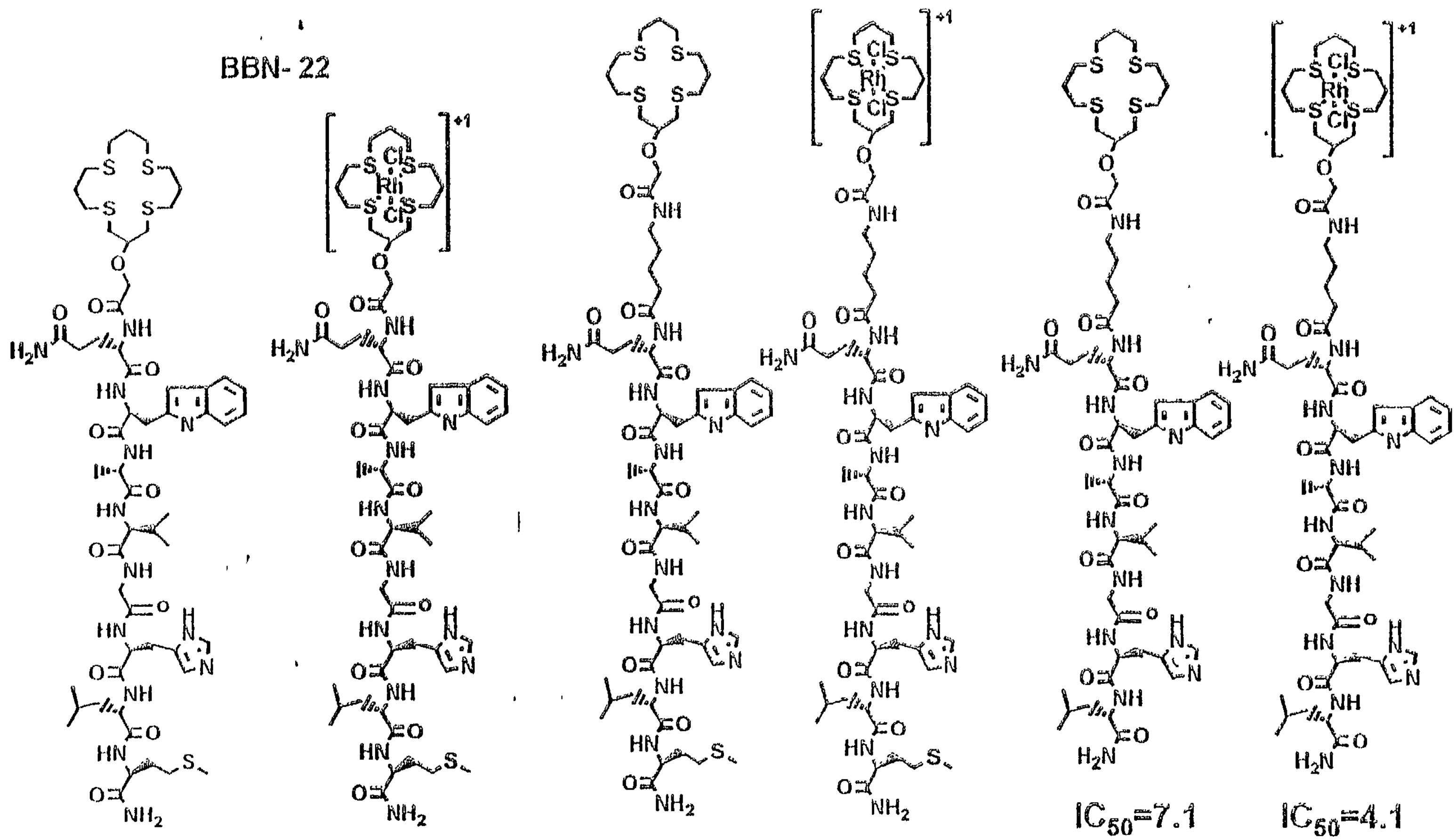


Figure 13

^{105}Rhodium Bombesin Analogues

BBN-22 BBN-37 BBN-61



$IC_{50}=3.0$ $IC_{50}=37.5$ $IC_{50}=2.0$ $IC_{50}=4.0$ $IC_{50}=7.1$ $IC_{50}=4.1$

Figure 14

^{105}Rh-BBN-61 Efflux Evaluation

Swiss 3T3 Cell Evaluation

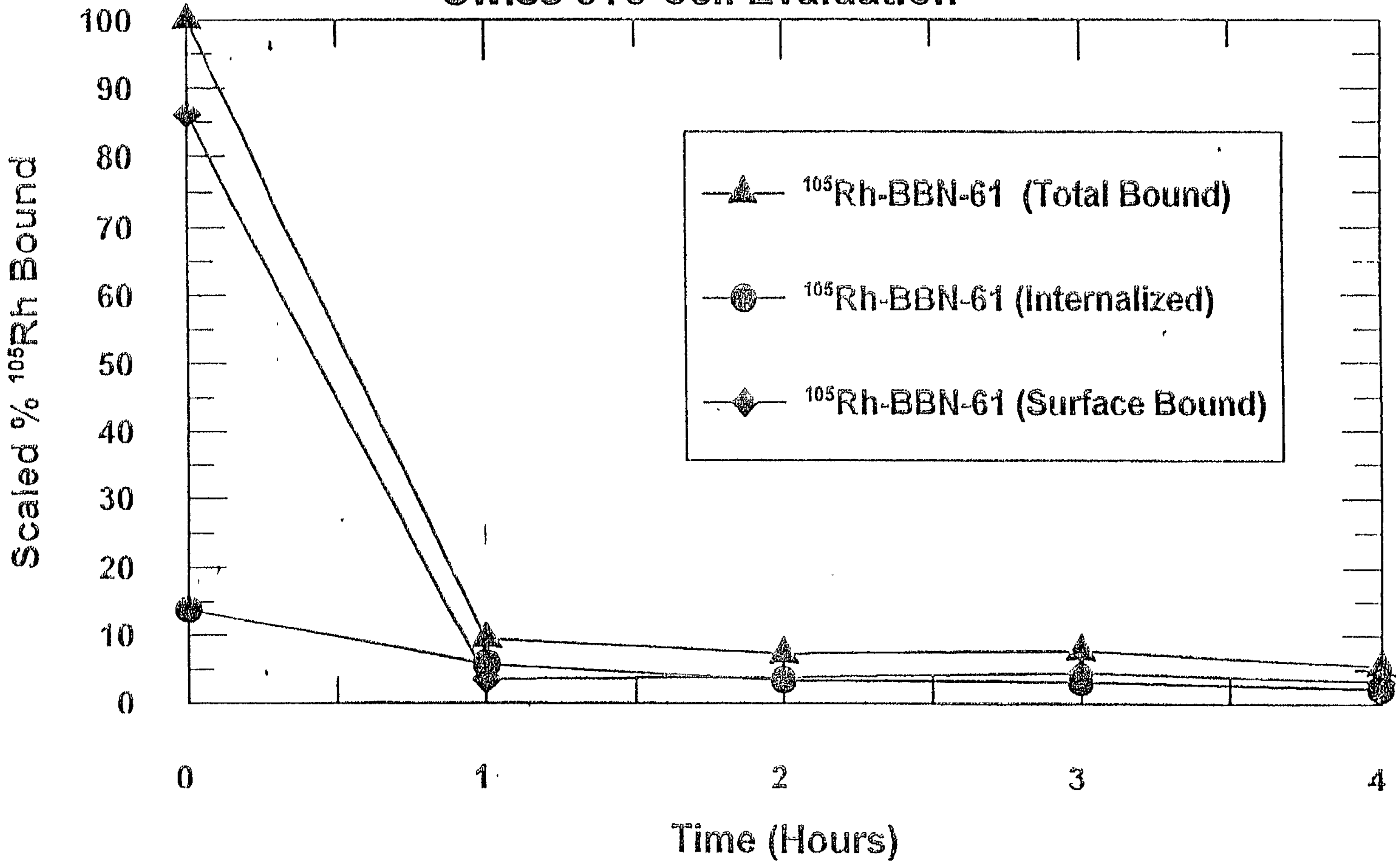


Figure 15

Efflux of ^{105}Rh-BBN-22 vs. ^{105}Rh-BBN-37 in Swiss 3T3 Cells
(Non-Normalized Data)

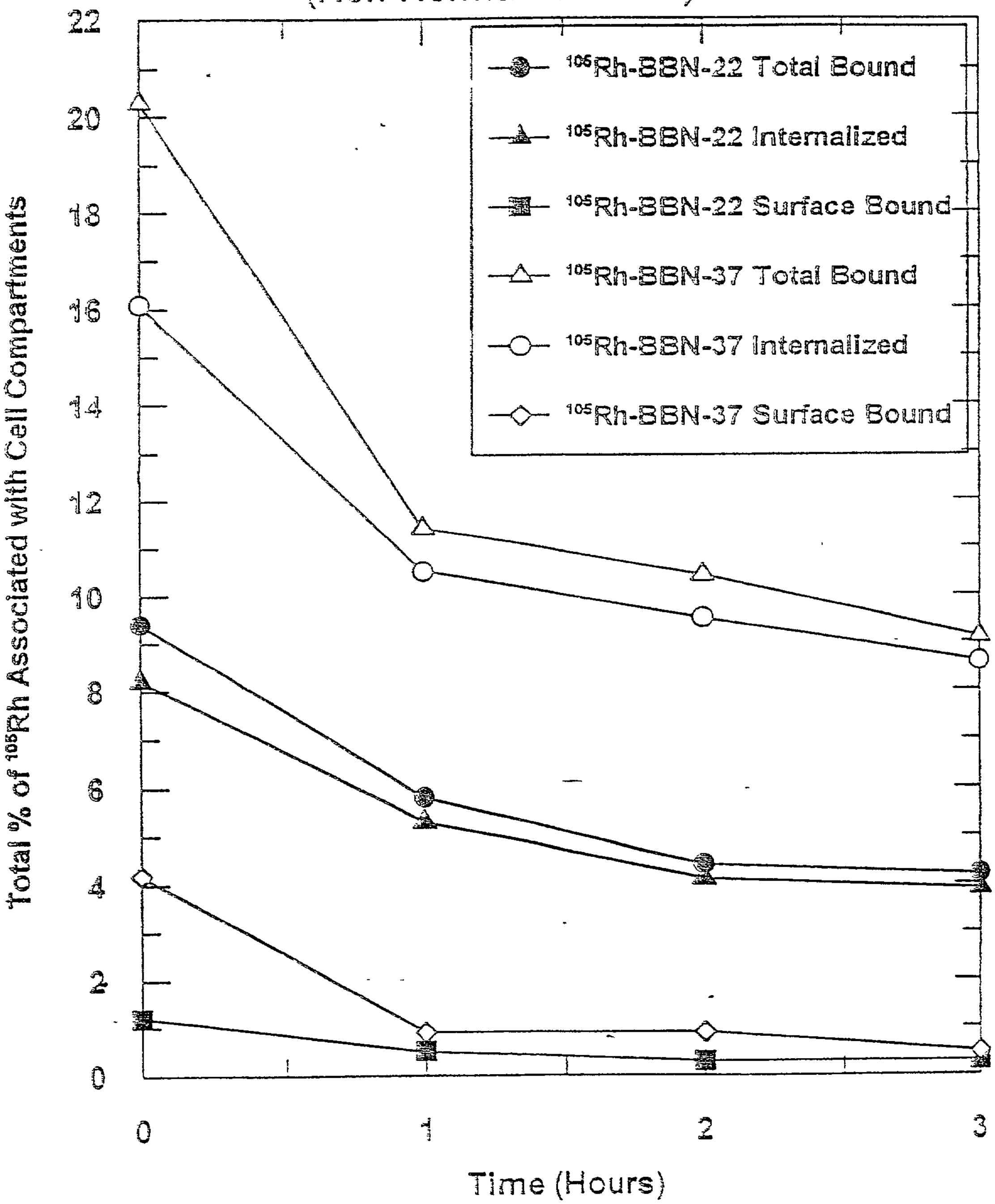


Figure 16

Pancreatic CA Cell Binding

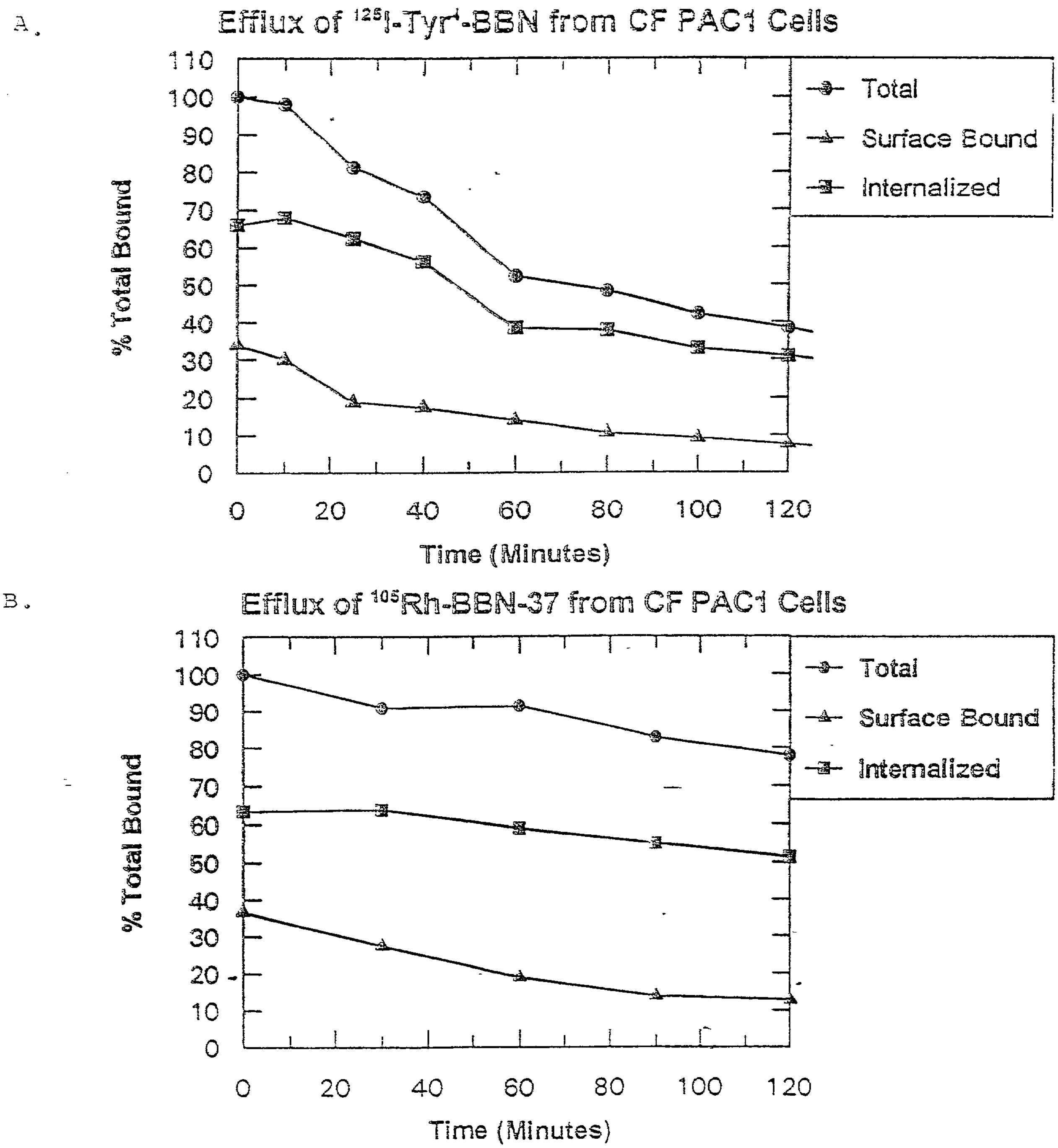
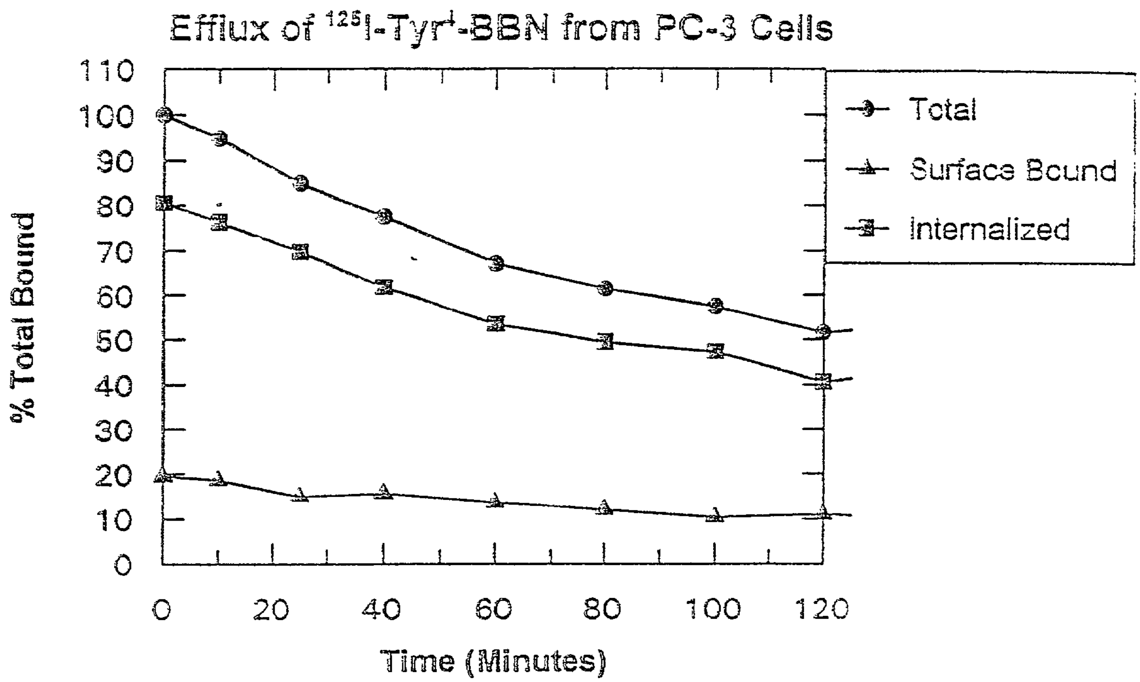


Figure 17

Prostate CA Cell Binding

A.



B.

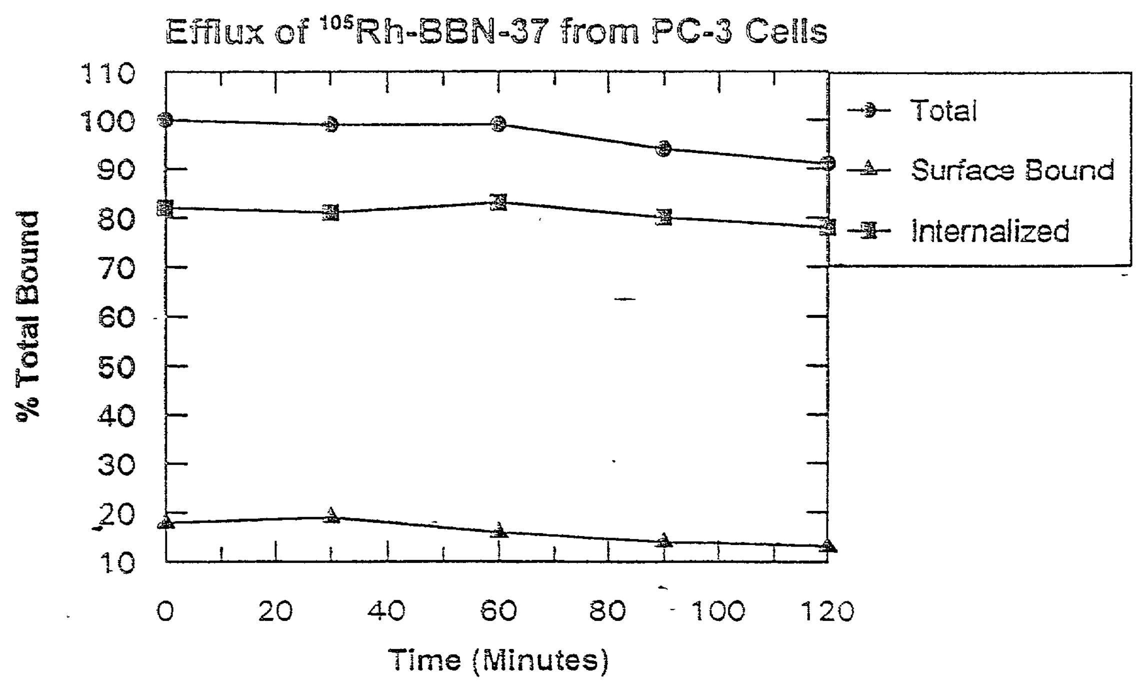
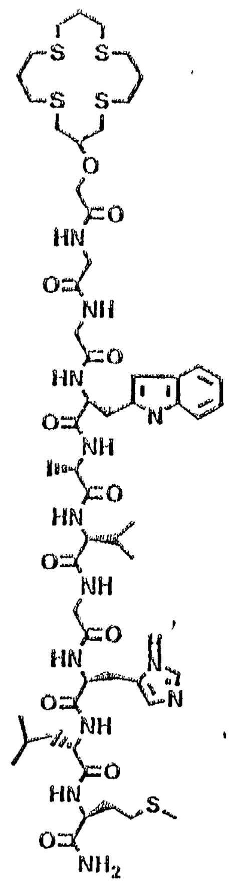


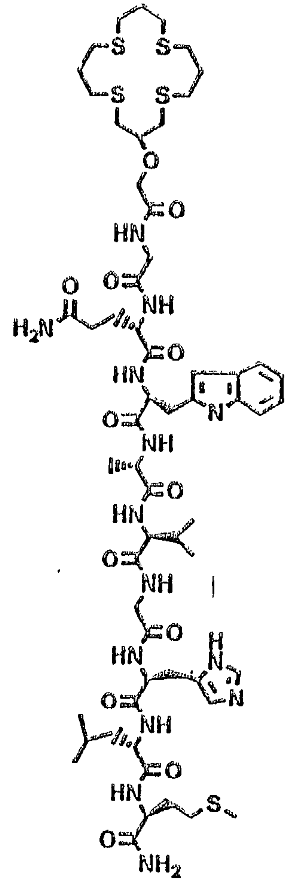
Figure 18

[16]aneS$_4$ Bombesin Analogues

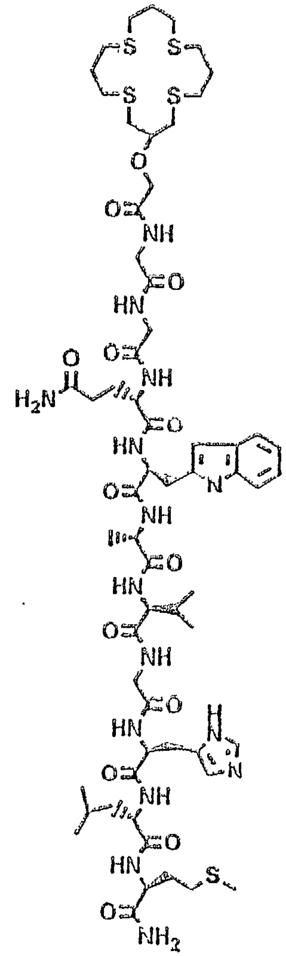
BBN-96



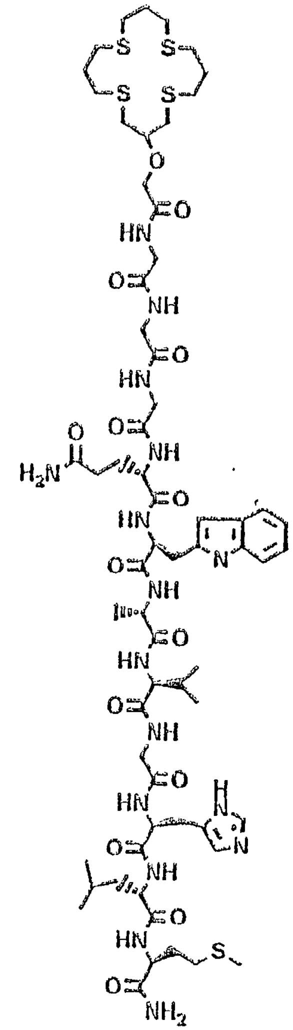
BBN-99



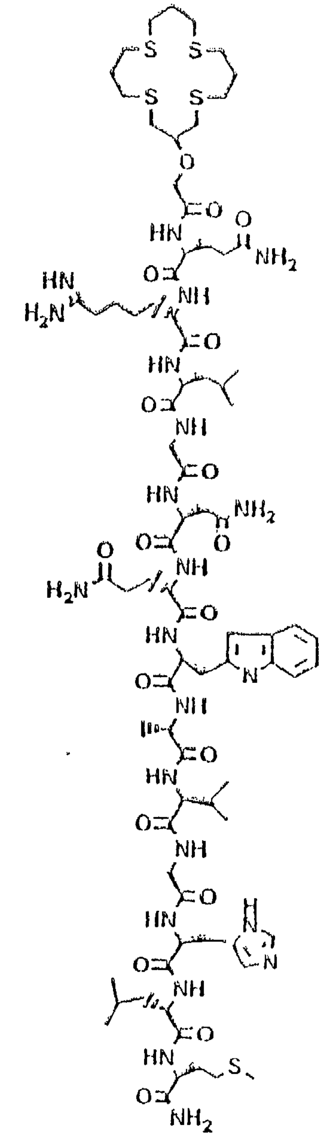
BBN-98



BBN-97



BBN-101

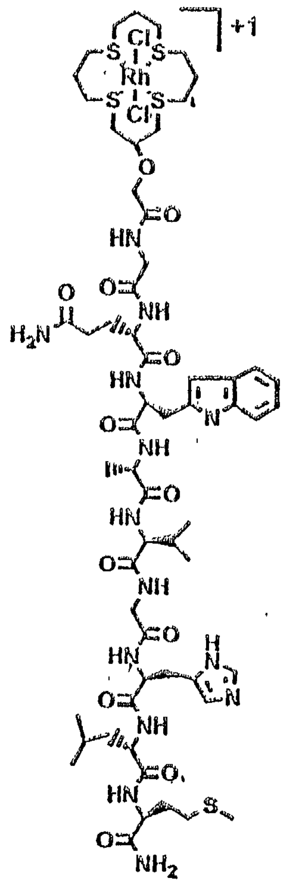


	BBN-96 IC_{50} (nM)	BBN-99 IC_{50} (nM)	BBN-98 IC_{50}(nM)	BBN-97 IC_{50}(nM)	BBN-101 IC_{50}(nM)
Swiss 3T3	18.4 ± 4.5	6.0 ± 0.5	3.3 ± 0.2	38.0 ± 11.7	1.2 ± 0.7
PC - 3	8.8 ± 1.8	3.3 ± 1.9	4.3 ± 2.2	6.9 ± 2.8	2.1 ± 0.5
CF PAC - 1	39.5 ± 10.7	4.8 ± 0.8	6.1 ± 3.3	14.2 ± 6.8	2.4 ± 0.9

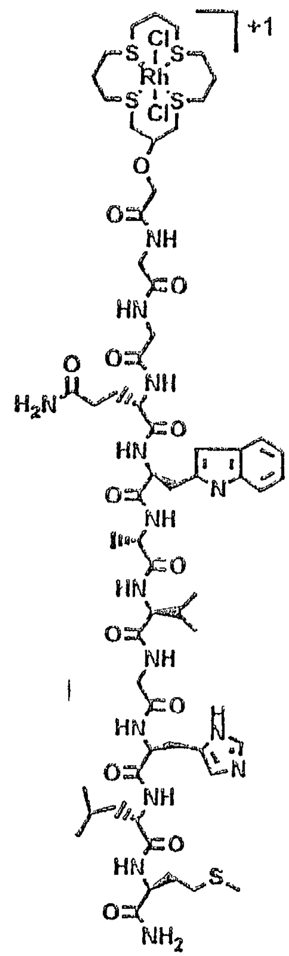
FIGURE 19

Rhodium-[16]aneS_4 Bombesin Analogues

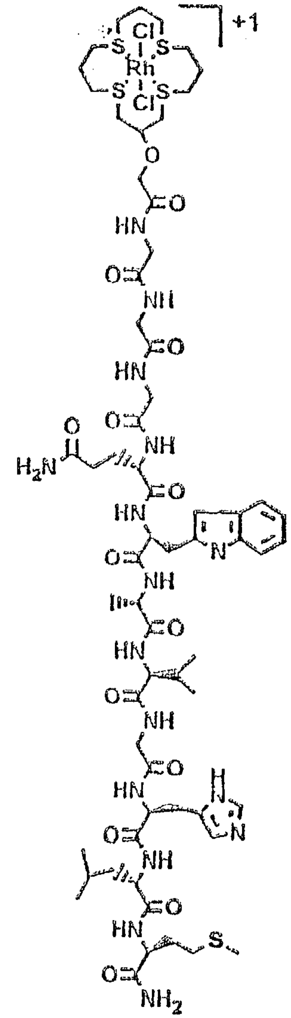
BBN-99



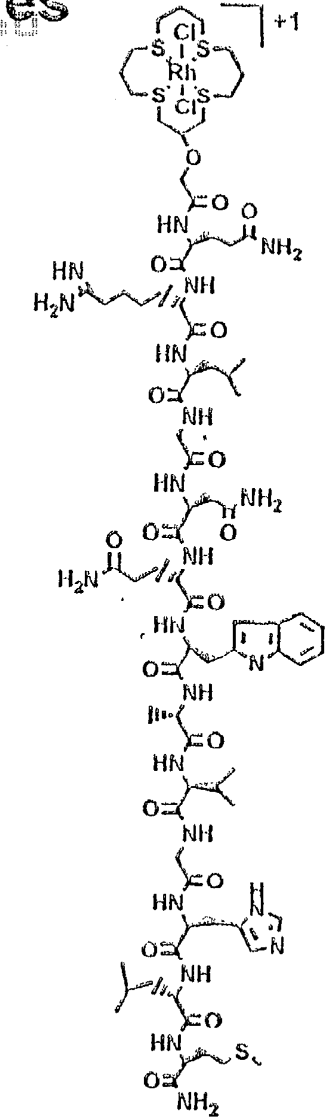
BBN-98



BBN-97

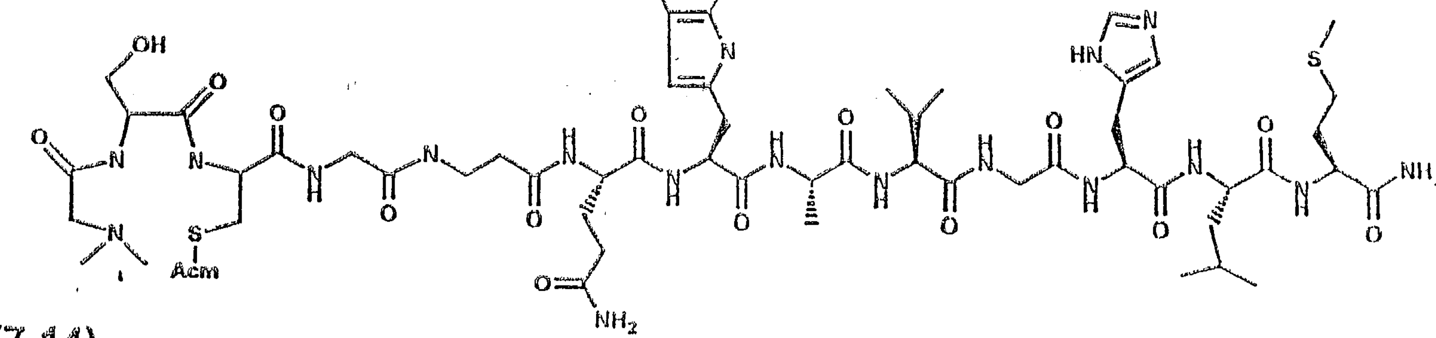


BBN-101

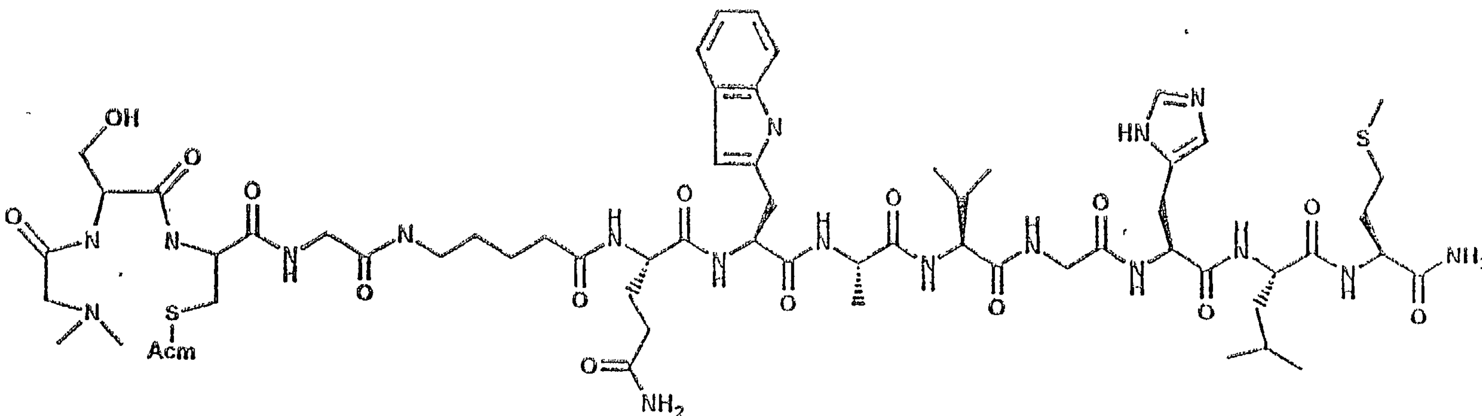


IC_{50} (nM)	BBN-99	BBN-98	BBN-97	BBN-101
Swiss 3T3	8.24 ± 0.67	2.84 ± 0.76	9.30 ± 1.55	5.42 ± 2.11
PC - 3	4.97 ± 0.60	1.21 ± 0.19	5.29 ± 092	2.32 ± 1.52
CF PAC - 1	4.64 ± 0.81	1.32 ± 0.12	3.70 ± 1.89	0.73 ± 0.83

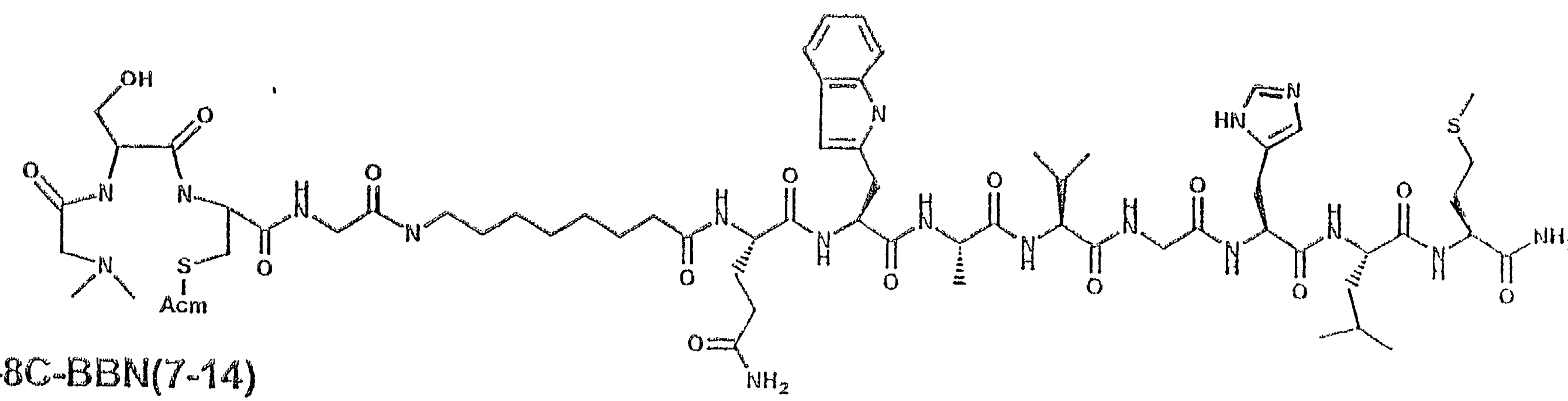
FIGURE 20



RP414-3C-BBN(7-14)



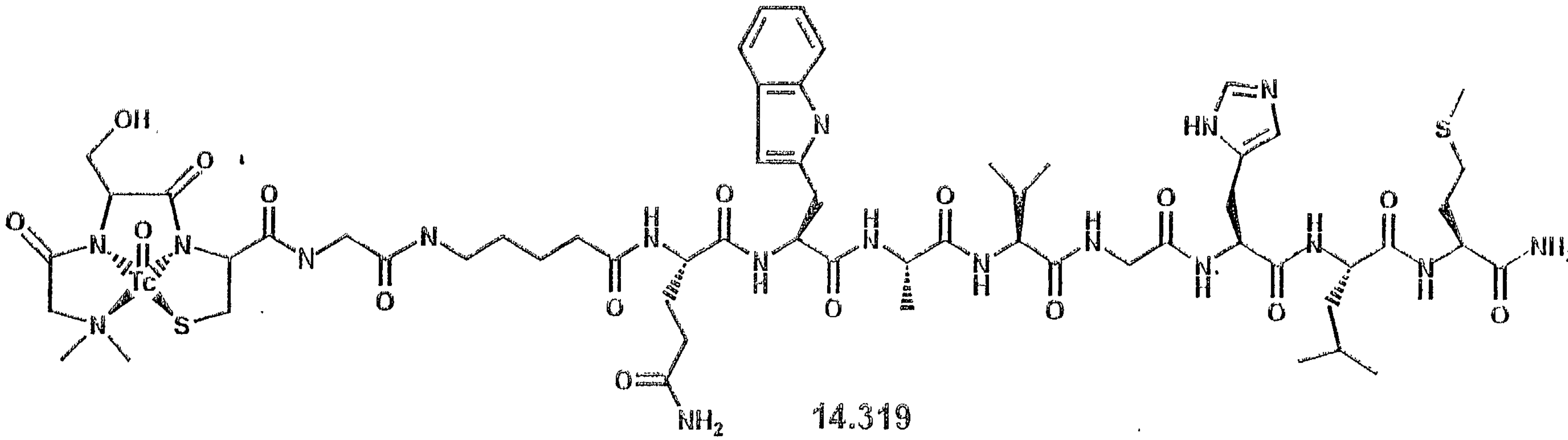
RP414-5C-BBN(7-14)

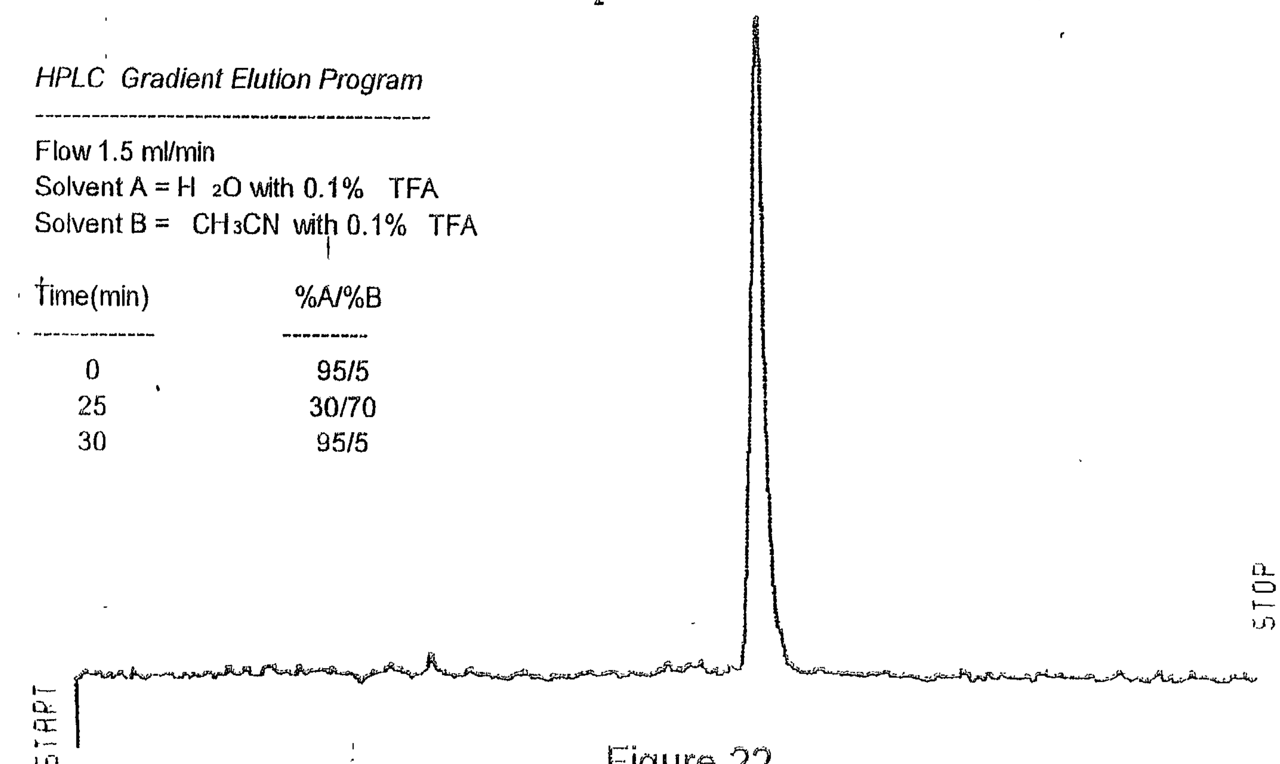


RP414-8C-BBN(7-14)

FIGURE 21

^{99m}Tc-BBN-122





HPLC Gradient Elution Program

Flow 1.5 ml/min
Solvent A = H_2O with 0.1% TFA
Solvent B = CH_3CN with 0.1% TFA

Time(min)	%A/%B
0	95/5
25	30/70
30	95/5

Figure 22

^{99m}Tc-BBN-122 Uptake in Human Prostate Cancer Cells

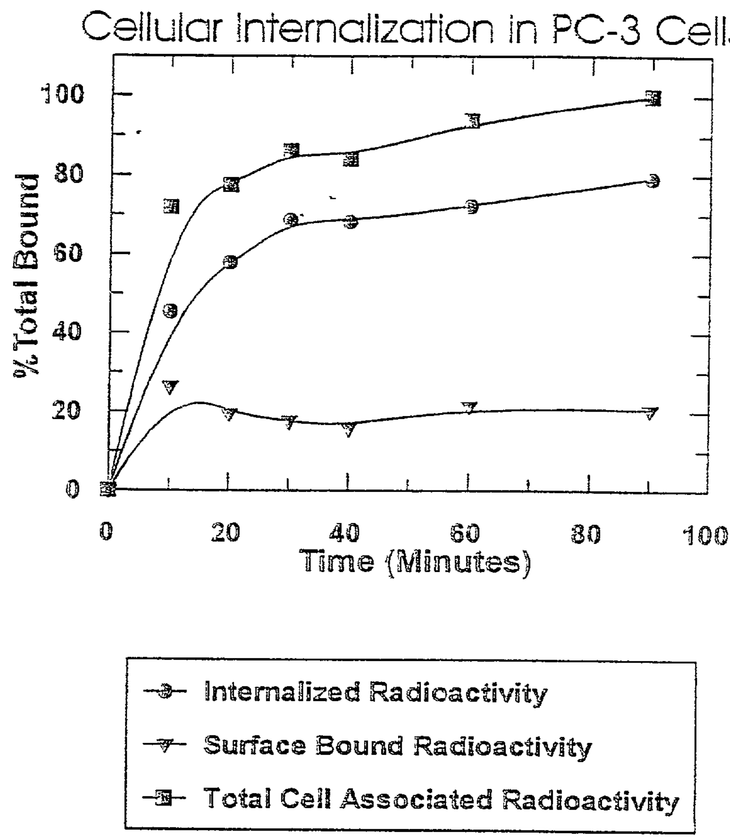


Figure 23

^{99m}Tc-BBN-122 Internalization in Human Pancreatic Cancer Cells

Cellular Internalization in CFPAC-1 Cells

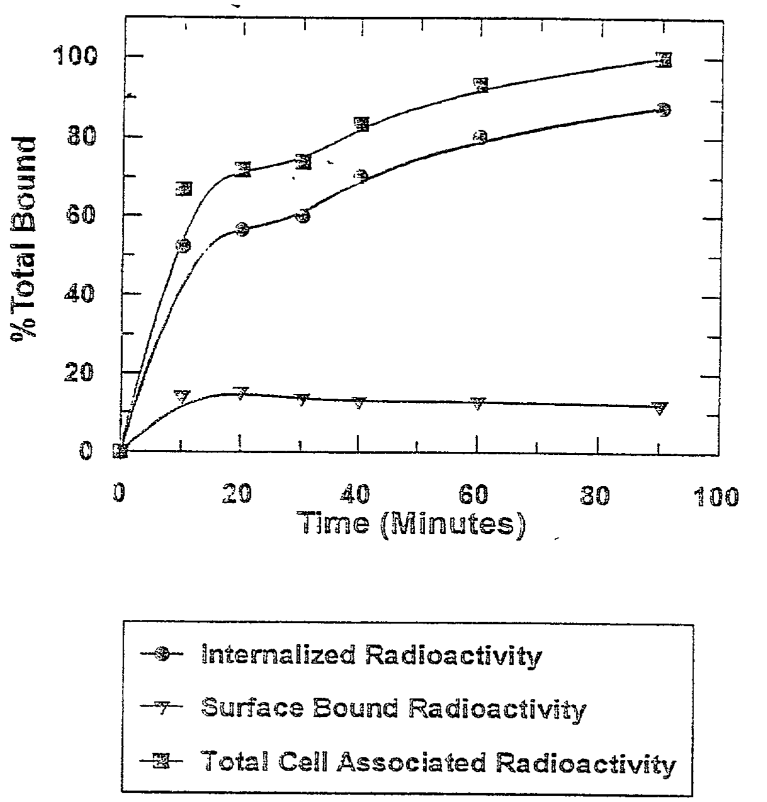


Figure 24

^{99m}Tc-BBN-122 Retention in Human Prostate Cancer Cells

Cellular Efflux from PC-3 Cells

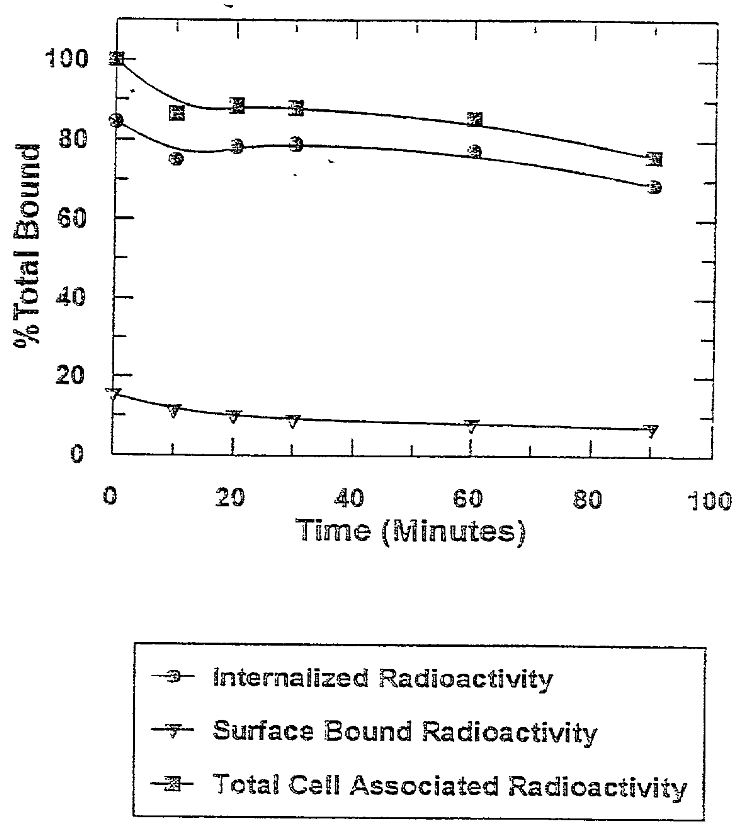


Figure 25

^{99m}Tc-BBN-122 Retention in Human Pancreatic Cancer Cells

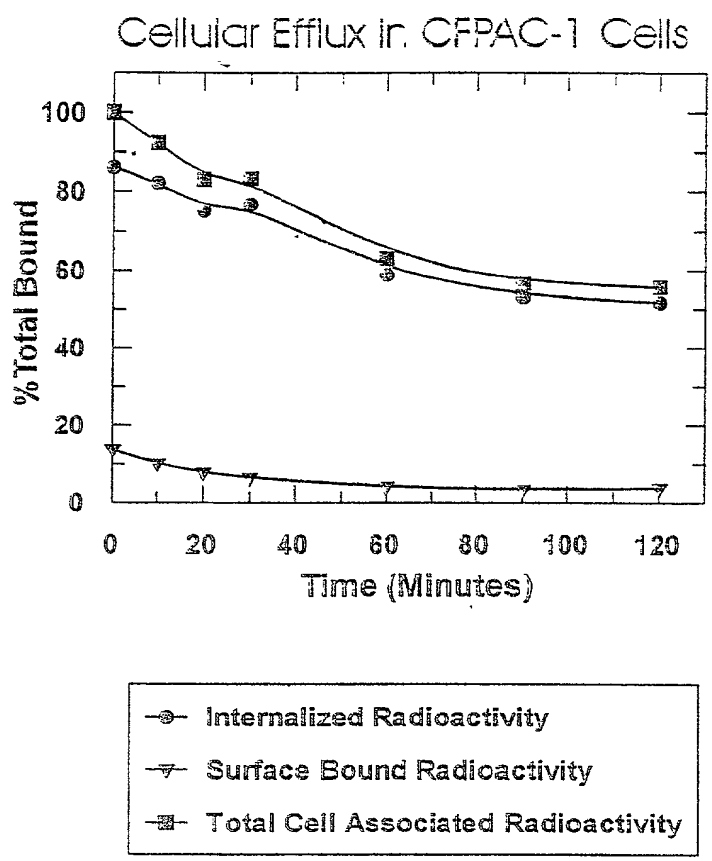
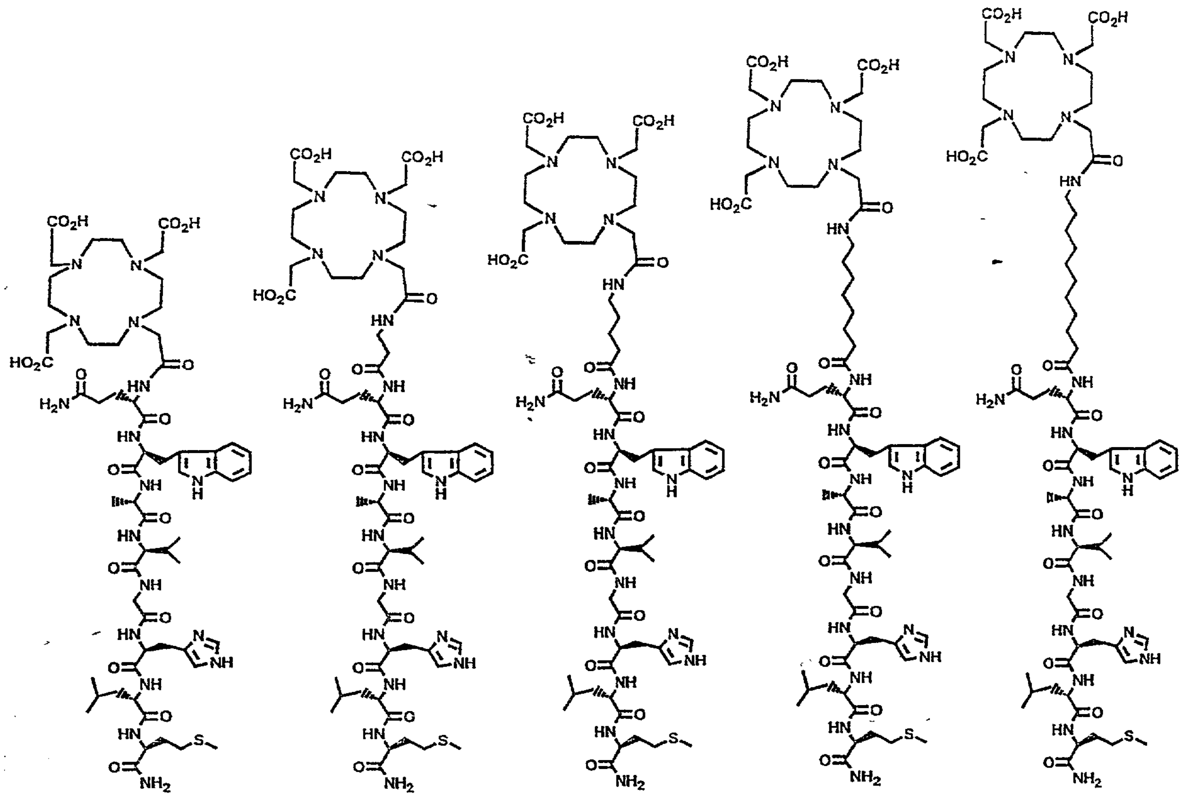


Figure 26

DOTA-BBN[7-14]NH_2 analogues.



DOTA-0-BBN(7-14)NH_2 DOTA-βAla-BBN(7-14)NH_2 DOTA-5Ava-BBN(7-14)NH_2 DOTA-8Aoc-BBN(7-14)NH_2 DOTA-11Aun-BBN(7-14)NH_2

FIGURE 27

HPLC chromatograms of (a) DOTA-BBN[7-14]NH_2 (λ = 280 nm) (b) In-DOTA-BBN[7-14]NH_2 (λ =280 nm) and (c) ^{111}In-DOTA-BBN[7-14]NH_2 (radiometric).

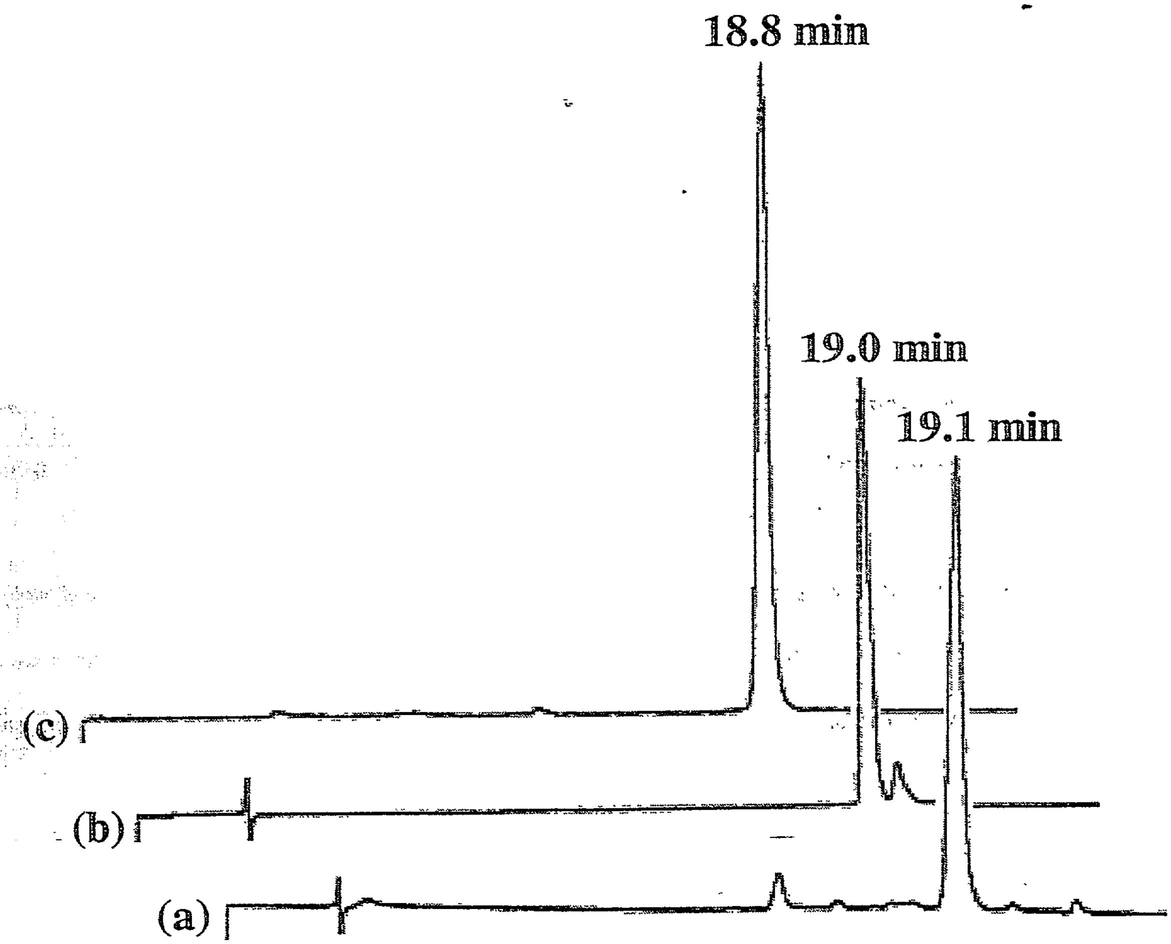


FIGURE 28

Competitive binding assay of In-DOTA-8-Aoc-BBN[7-14]NH_2 vs. ^{125}I-Tyr^4-BBN in PC-3 cells.

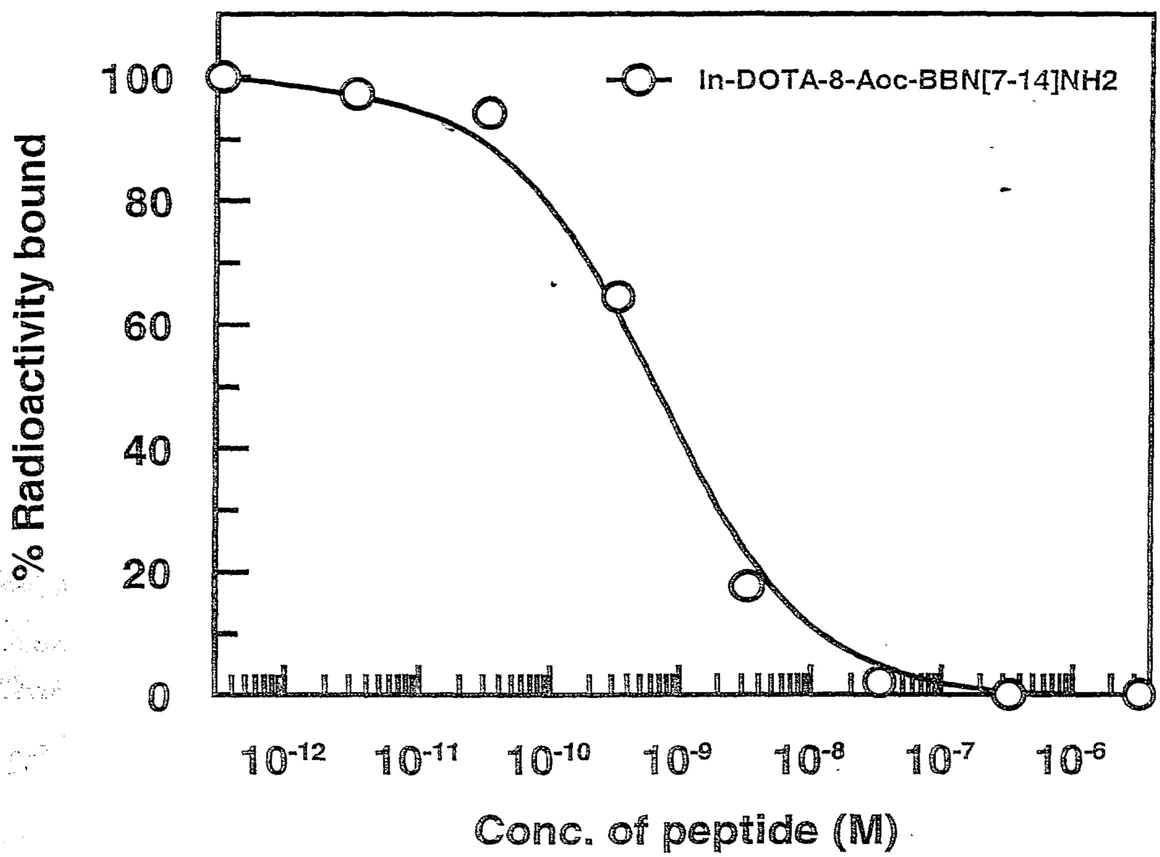


FIGURE 29

Internalization of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in PC-3 cells.

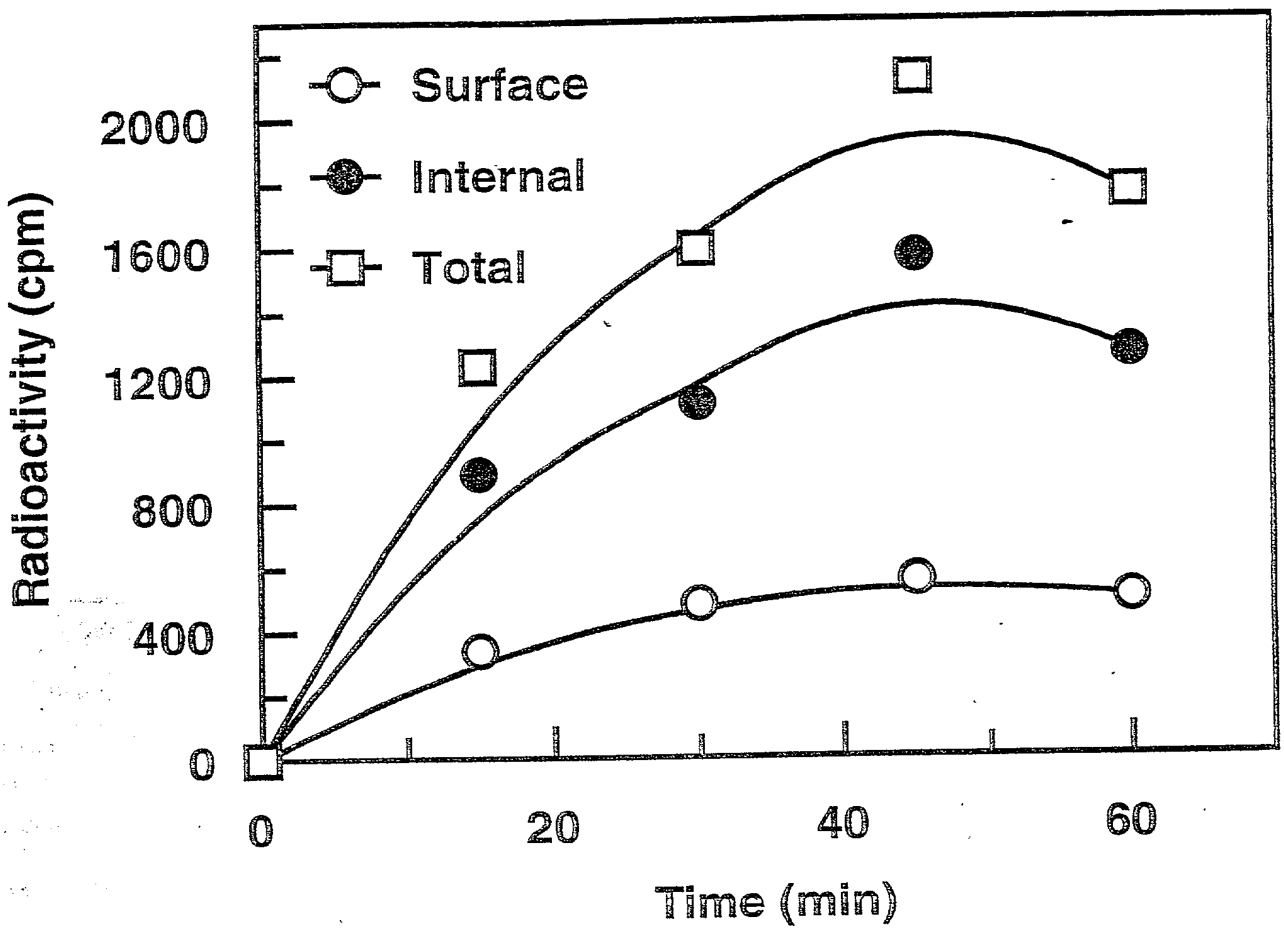


FIGURE 30

Efflux of ^{111}In-DOTA-8-Aoc-BBN[7-14]NH_2 in PC-3 cells.

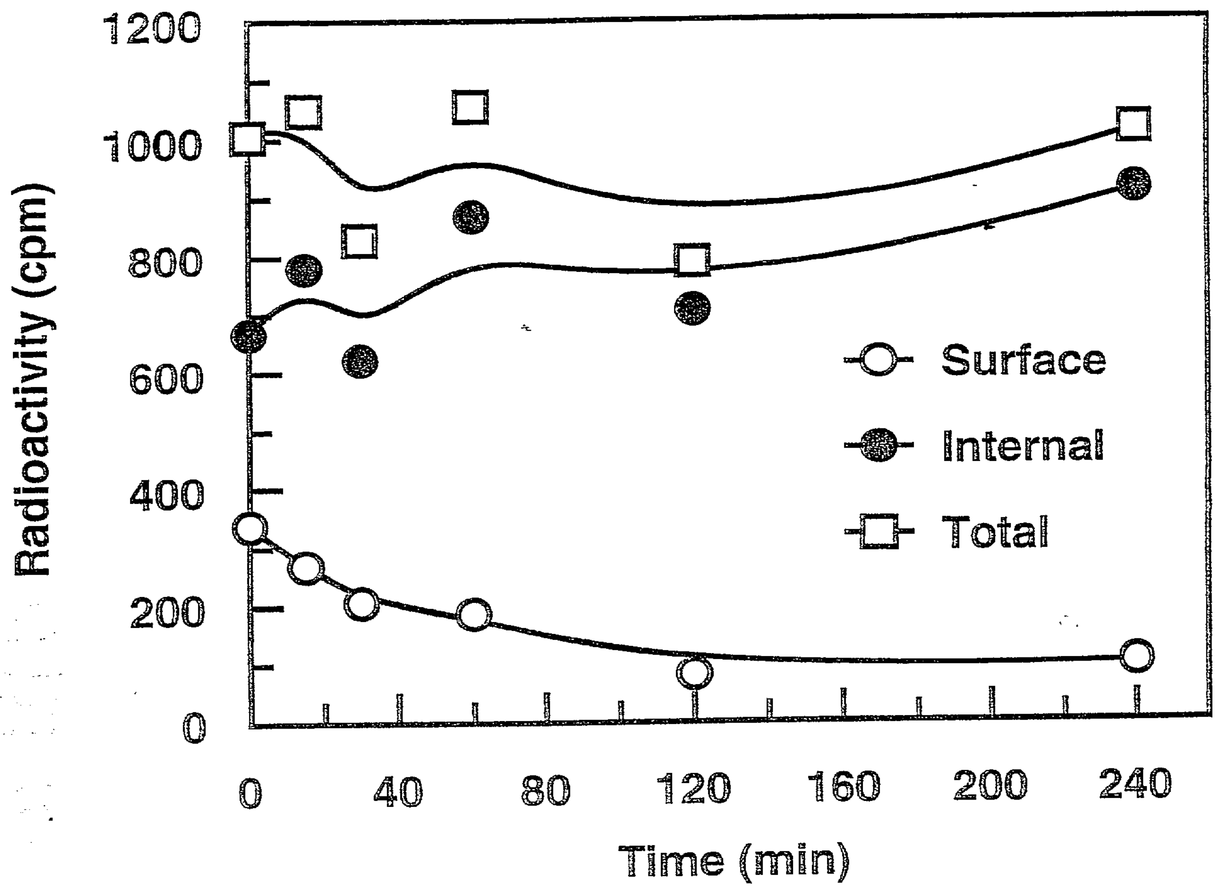


FIGURE 31

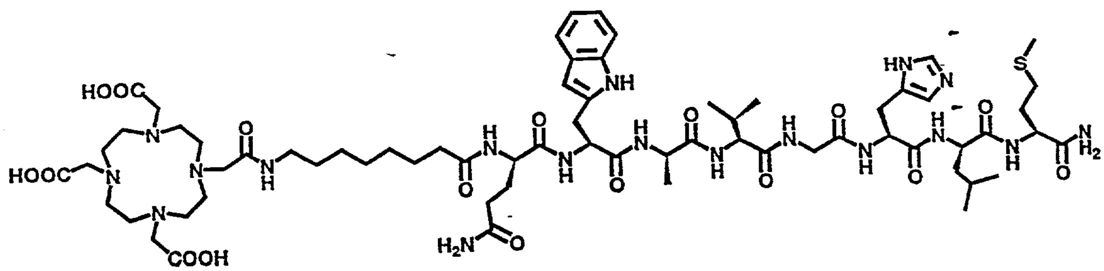

HOOC
HOOC
COOH
N
N
N
N
O
N
H
O
N
H
H
N
O
H₂N
O
N
H
NH
O
H
N
O
N
H
O
N
O
N
H
HN
N
O
H
N
O
N
H
S
O
N
H₂

Figure 32